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THE UNIVERSITY OF ALBERTA

AN ANALYSIS OF THE RESPONSE OF RABBIT PORTAL VEIN TO HISTAMINE

by

Ⓒ KATHLEEN MARY MACLEOD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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TO STEVE

For his humor, tolerance, helpfulness and encouragement.

ABSTRACT

Histamine produces dose-dependent contractions of isolated spiral strips of rabbit portal vein. The response to histamine is unaffected by atropine or indomethacin, suggesting that neither acetylcholine nor prostaglandins play a role in the response to this agonist. Treatment of tissues with cocaine, or 6-OHDA or induction of tachyphlaxis to tyramine, all of which reduce or abolish the response to tyramine, have no effect on the response to histamine. These results suggest that histamine is not acting indirectly through the release of an endogenous transmitter in this preparation. However the receptor for histamine does not appear to be either a typical H_1 or H_2 receptor; the histamine response is not blocked by the H_2 antagonist metiamide, and is blocked only by high concentrations of the H_1 antagonists, chlorpheniramine, diphenhydramine and antazoline. The most effective antagonists of the histamine response are the α-adrenergic blocking agents, phentolamine, azapetine and dibozane, which also antagonize the response to noradrenaline in this preparation.

5-hydroxytryptamine (5-HT) also elicits dose-dependent contractions of rabbit portal vein. The responses to high concentrations of this agonist are insensitive to methysergide and 6-hydroxydopamine (6-OHDA), but are blocked by phentolamine. Induction of desensitization to 5-HT results in a concomitant depression of the responses to equi-active doses of histamine and noradrenaline. The maximum responses to 5-HT and histamine, but not to noradrenaline are also depressed by this treatment. High concentrations of histamine, but not 5-HT, antagonize the response to noradrenaline in the presence of cocaine.

The responses to noradrenaline, histamine and 5-HT are all

depressed by the irrevisible antagonist phenoxybenzamine. Noradrenaline and phentolamine cross-protect the responses to all three angonists against POB blockade. Even high concentrations of histamine do not provide good protection of the responses to itself or other agonists, while the protection provided by 5-HT appears to have a significant non-specific component.

The most satisfactory explanation of these results is that noradrenaline, 5-HT and histamine are interacting at a common, non-specific receptor, at which noradrenaline behaves as a full agonist, and histamine and 5-HT as partial agonists. The receptor for these agonists in portal vein bears close resemblance to the α-adrenoceptor and may represent a furthur subtype of this receptor.

The responses to histamine, 5-HT, tolazoline and betazole all show sensitization with time, while the responses to noradrenaline and acetylcholine do not. The increased sensitivity of portal vein to histamine appears to be selective, since the response to acetylcholine is not increased after exposure of the tissue to histamine. The sensitization is unaffected by atropine or indomethacin, but it is reduced in reserpinized tissues, suggesting that it has an adrenergic component. It also appears to be reduced after treatment of tissues with methysergide.

Histamine causes a 6-OHDA-sensitive increase in the efflux of $[^3H]$-noradrenaline from rabbit portal vein, and 6-OHDA treatment of isolated tissues abolishes the increase in sensitivity to histamine. The histamine-induced increase in efflux of $[^3H]$-noradrenaline is also reduced by cocaine; this agent reduces the sensitization to histamine in a reversible manner.

These results imply that although the major component of the histamine response occurs through a direct interaction with a non-specific amine receptor, the sensitization component of this response occurs indirectly through the release of noradrenaline. The mechanism by which this release occurs is not clear at the present time, nor is the involvement of methysergide in the inhibition of sensitization.

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INTRODUCTION

I GENERAL INTRODUCTION

Histamine (β-imidazolylethylamine, Fig. 1) was first synthesized by Windaus and Vogt in 1907, but it was not until 1910 that Dale and Laidlaw demonstrated its dramatic and widespread physiological effects. Since then, histamine has been shown to have profound actions on all parts of the cardiovascular system, and this thesis is concerned with a component of these actions which is still quite poorly understood: the effects of this compound on the smooth muscle of veins.

Almost 70 years have passed since the first description of the biological activity of histamine. The demonstration of its occurrence as a natural constituent of body tissues did not occur until 17 years later (Best et al., 1927). Since then, much information has been obtained about its broad distribution, as well as its synthesis, storage, metabolism and release. Much less is known about the physiological function of this ubiquitous compound, however. Release of histamine from its mast cell storage sites has been shown to play an important role in the process of anaphylactic shock, although it is not the only substance involved (Douglas, 1975). The only regulatory function of histamine which has been convincingly demonstrated, though, is in the control of gastric acid secretion (Kahlson and Rosengren, 1968). Other suggestions that histamine is involved in the regulation of the microcirculation (Schayer, 1963) and of tissue growth and repair (Kahlson and Rosengren, 1971), as well as mediating the active component of vasodilatation (Beck, 1965), seem less well established. A major hindrance to the effort to show a broader physiological function

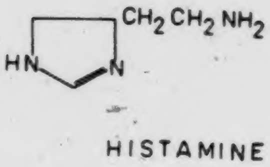


FIG. 1 The chemical structure of histamine.

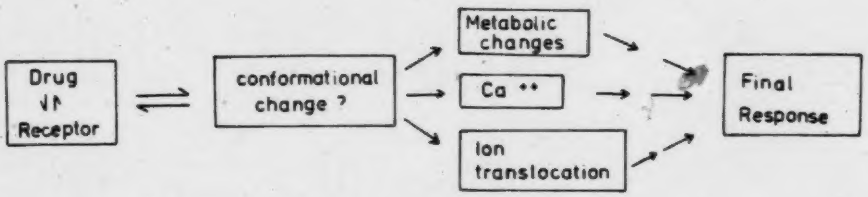

Drug
Receptor
conformational change ?
Metabolic changes
Ca ++
Ion translocation
Final Response

FIG. 2 Schematic diagram illustrating some of the events leading to smooth muscle contraction (After DeRobertis, 1975).

for this compound has been the inability to demonstrate, either pharmacologically or histochemically, the presence of peripheral histaminergic nerves, although there is some suggestion that these may be present in the central nervous system (Schwartz, 1977). The technical difficulties involved in determining a regulatory role for histamine in most of these processes are still quite severe however, and the demonstration of the widespread distribution of specific receptors for this compound suggests the possibility that histamine does, in fact, have a physiological function which has not yet been recognized.

The actions of histamine in the cardiovascular system in particular have been examined in some detail, and as outlined below, most types of vascular smooth muscle and cardiac muscle respond to this compound, both in vivo and in vitro.

In most animals, except the rabbit, injection of histamine results in a dose-dependent fall in blood pressure (Brimblecombe et al., 1974; Black et al., 1975; Powell and Brody, 1976), although in some species, notably the cat and the guinea pig, the initial fall is followed by a secondary increase probably due to catecholamine release from chromaffin tissue (Owen, 1977). The response of the rabbit is atypical - histamine causes biphasic changes in blood pressure in this species, which consist of an initial pressor response followed by a more sustained depressor response, both of which are a result of a direct action of histamine (Naranjo and Naranjo, 1958; Parsons and Owen, 1973).

When given systemically, histamine causes a decrease in total peripheral resistance which probably accounts, in large part, for the vasodepressor effects of this compound (Johnston and Owen, 1977;

Thermann _et al._, 1975). This occurs through vasodilatation of some peripheral vessels, but it is apparent that vasoconstriction of other peripheral blood vessels can also occur, depending on the species being examined (Johnston and Owen, 1977).

Histamine also has profound effects on the isolated heart and on some isolated blood vessels. It causes contraction of most large arteries and veins examined _in vitro_ (Somlyo and Somlyo, 1970), although dilatation has occasionally been demonstrated. The most common effect of histamine on the heart is to increase both the rate and force of contraction (Trendelenburg, 1960; Bartlet, 1963, Flacke _et al._, 1967).

It is clear from the foregoing remarks that the widespread actions of histamine within the cardiovascular system are diverse, depending on the species and the tissue being examined. Although much is known about the overall response of animals to histamine, less information is available concerning the response of individual blood vessels, particularly veins, to this compound. We therefore decided to examine in detail the response of isolated venous smooth muscle to histamine, and because of its importance to the cardiovascular system, we chose to study the histamine response of the isolated hepatic portal vein. As mentioned earlier, histamine is believed to act through receptors, and in the next section the evidence for the existence of these structures, and some of their properties, are reviewed.

II RECEPTORS

The concept that there exists a specific area on the surface of macromolecules or cells to which substances can combine to bring about their action, arose independently in two different disciplines around the turn of the century. Emil Fischer demonstrated the specificity of the enzyme-substrate interaction in 1894, and in his now famous analogy suggested that the substrate fits into an area of the enzyme the way a key fits into a lock. Paul Erlich, in considering immunological phenomena, found that an introduced molecule must often bind to a specific area of an endogenous molecule in order to be active, and he defined that area of the endogenous molecule as the receptor. This concept was extended to the field of pharmacology by J.N. Langley in 1905. After examining the effects of nicotine and curare on the neuromuscular junction, he concluded that nicotine must have its action by combining with a 'receptive substance' on the muscle cell, which then transferred the stimuli it received to the contractile apparatus of the muscle (Langley, 1905). Since that time, it has been widely accepted that many other drugs and endogenous hormones also produce their actions by combination with a specific structure located on or within a cell. Although the term 'receptor' has been used in other contexts since it was coined, in general the receptor is taken to be only that site or structure with which an agonist combines to produce the characteristic response of the cell to that agonist, as proposed by Furchgott (1964).

A. Evidence for Receptors

In general, there are three major reasons why drugs are believed to act through receptors (Rang, 1971). First, most drugs are active in relatively low concentrations, some being active in concentrations as low as 10^{-12}M. Secondly, evidence exists to imply chemical specificity in the action of drugs, such as the differential potencies of optical isomers, and the availability of specific drug antagonists. And thirdly, there is biological specificity in the action of drugs, since not all drugs are active on all cells or tissues.

The above points form convincing evidence for the existence of receptors, but this evidence is indirect. The isolation of receptors and direct demonstration of their nature and properties would be far more convincing, but in most cases this has so far proved impossible. The reason for this reflects a fundamental characteristic of these entities - it is impossible to subject them to the isolation procedures used so commonly in enzymology because they function only as transducers of messages across the cell membrane while having no recognized innate catalytic activity. There have been in the past many attempts to isolate receptors by other methods, the most common of which was the use of labelled high affinity or irreversible antagonists or high-affinity agonists to bind to receptors, which were then subjected to isolation procedures. For the most part these attempts failed for a variety of reasons, the most important of which was that most tissues contain very few receptors relative to other sites at which the agents used for isolation could also bind (May _et_ _al_., 1967; Moran _et_ _al_., 1967; Terner _et_ _al_., 1971). Although this difficulty seems to have been overcome in the case of the nicotinic cholinergic receptor, most information concerning the characteristics of receptors for other substances, including the receptor for histamine, and the manner in which drugs interact with them, must come from the classical pharmacological methods. These techniques are all indirect and in most cases measure the response of various isolated smooth muscles to agonists in the presence and absence of antagonists. Clark in 1937, Paton in 1961 and Rang in 1973, among others, proposed various models describing drug-receptor interactions, based on the results of these types of experiments. Although these models are necessary and valuable for their predictive potential, none of them has been either discarded or unequivocally shown to be correct. This illustrates the grave limitations inherent in trying to determine events occurring at the molecular level from such a gross phenomenon as smooth muscle contraction. These difficulties arise because, as shown in Fig. 2 (DeRobertis, 1975) the interaction of drug with receptor is only the first step leading to the response of the muscle, and alterations in the measured response, as well as reflecting events at this step, could also reflect changes occurring at any of the other steps. The most widely accepted means of circumventing this problem is the use of the so-called 'null method' in which equiactive doses of agonist are compared before and after treatment of the tissue with antagonist. In this case, it is hoped that the contribution of events following the drug-receptor interaction to the alteration in the final response is kept to a minimum (Waud, 1968). This method has been widely used to provide information about drug-receptor interactions, including the specificity of these interactions, as described in the next section.

B. The Histamine Receptor

1. Specificity

Receptors which are specific for certain agonists, including histamine, have been demonstrated in a number of ways. The existence of specific antagonists which block the action of one agonist while having little affect on the response to another, has already been mentioned. In 1947 Schild introduced a method of quantifying the blocking activity of competitive antagonists, with the use of the pAx value. The pAx is the negative logarithm (to the base 10) of the concentration of an antagonist necessary to shift the dose-response curve to an agonist x log units to the right (Schild, 1947a, b). The most commonly used pAx value is the pA_2. These values are a function only of the receptor and the antagonist, and thus when two agonists are acting at the same receptor, an antagonist will have the same pA_2 against both agonists. Schild (1974b) went on to demonstrate that the histamine antagonists available at that time were indeed specific for histamine, since pA_2 values for various antagonists against histamine were different from those found against other agonists.

Another means of differentiating receptors is the receptor protection technique (Furchgott, 1954). This method makes use of the ability of agonists and antagonists which act at a receptor to protect that receptor against blockade by a non-specific irreversible antagonist, while receptors for other agonists are blocked. This method has been used to demonstrate the existence of specific histamine receptors in a number of tissues. High doses of histamine were found to protect the histamine response but not the responses to other agonists such as 5-hydroxytryptamine (5-HT), noradrenaline and acetylcholine in rabbit aorta, guinea pig ileum and cat splenic strip (Furchgott, 1954; Innes, 1962a; Ariens _et_ _al_., 1960). Similarly, the histamine response in guinea pig ileum could be protected by histamine antagonists, which did not protect the responses to other agonists (Kenakin, 1975).

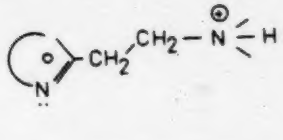
A third means of differentiating receptors is to desensitize the response to one agonist and find if the response to another agonist is also reduced. Desensitization occurs when a tissue is exposed to a high concentration of agonist for a long period of time, and can take two forms. One of these is non-specific and results in responses to all agonists being depressed, while the other appears to be receptor specific and results in a reduction of the responses of only those agonists acting at that receptor (Rang and Ritter, 1970). Desensitization of the response to 5-hydroxytryptamine in spleen strips does not result in cross-desensitization of the histamine response (Innes, 1962b), while the noradrenaline response is not cross-desensitized when the histamine response is desensitized in atria (Dean, 1968), indicating that at least in these tissues, histamine is acting at a different receptor than 5-HT and noradrenaline.

2. Classification of Histamine Receptors

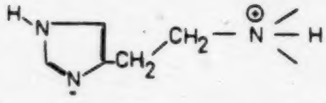
Histamine antagonists have been available since the late 1940's, and since then receptors specific for histamine have been shown to be widespread. It soon became apparent however, that the available antihistamines were not effective at blocking the histamine response in every tissue. The response to histamine in tissues such as guinea pig ileum and bronchial smooth muscle could be antagonized competitively by agents such as diphenhydramine and chlorpheniramine (Arunlakshana and Schild, 1959). However, these antagonists were ineffective against the histamine response in rat uterus, mammalian heart and on gastric acid secretion, although histamine did not appear to be acting at a receptor for another agonist in these tissues (Ash and Schild, 1966; Trendelenburg, 1960; Ashford *et al.*, 1949). In 1966, Ash and Schild proposed that there were at least two types of histamine receptors, after examining the blocking activity of the available histamine antagonists and the agonist activity of a series of histamine derivatives. They called the receptors which are competitively blocked by the classical anti-histamines, such as those in guinea pig ileum and bronchial smooth muscle, 'H_1' receptors. In 1972, Black and his co-workers succeeded in synthesizing a histamine antagonist, burimamide, which was an effective competitive antagonist of the histamine response in rat uterus and guinea pig atrium, as well as of the histamine-induced gastric acid secretion in rats and dogs (Black *et al.*, 1972). The histamine receptor in these tissues was called an 'H_2' receptor, and so far studies with H_1 and H_2 antagonists and with histamine analogs have indicated that no further sub-division of histamine receptors is necessary. It has since been reported that some H_1 antagonists can, in fact, block the H_2 receptor. McNeill and Verma (1974b) found that promethazine, an H_1 antagonist, could antagonize the histamine response in guinea pig atria, which is mediated by an H_2 receptor, but the blockade produced was non-competitive and occurred only at high doses of antagonist.

3. Structure-Activity Relationships and Relation of H_1 and H_2 Receptors

The availability of histamine analogs with varying degrees of activity at H_1 and H_2 receptors has enabled the determination of structure-activity relationships at H_1 and H_2 receptors, as well as facilitating the differentiation of these receptor types (Jones, 1966; Black *et al.*, 1972; Durant, Ganellin and Parsons, 1975). On the basis of these studies Durant *et al.* (1975) have determined the chemical requirements which are necessary for activity at each receptor subtype (Fig. 3). They found that for activity at either receptor the side-chain requirements were the same: The side-chain must be cationic and the nitrogen should not be completely substituted. The ring



H_1- RECEPTOR



H_2- RECEPTOR

FIG. 3 Structural requirements for activity at histamine receptors. (After Durant et al., 1975).

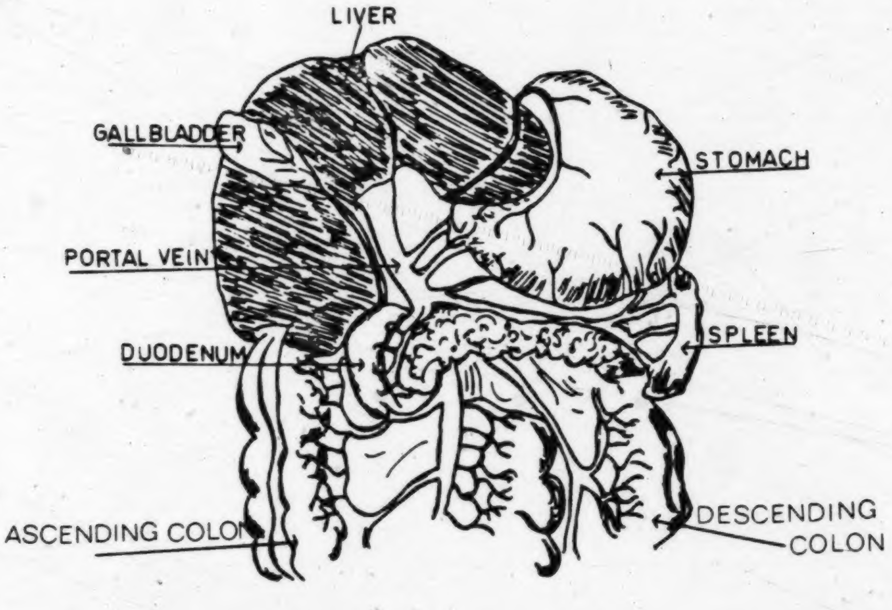

LIVER
GALLBLADDER
STOMACH
PORTAL VEIN
DUODENUM
SPLEEN
ASCENDING COLON
DESCENDING COLON

FIG. 4 Diagram illustrating the location of the portal vein in man and in other species.

requirements were different however; for activity at H_2 receptors an imidazole ring was required, with a hydrogen atom bound to the ring nitrogen in the 3 position, suggesting to these workers that at the H_2 receptor histamine might function as a proton transfer agent. On the other hand, for H_1 activity an imidazole ring was not a necessity, as long as the ring which was present could rotate freely and contained a nitrogen atom with a lone electron pair.

It has been generally assumed that the two histamine receptor subtypes bear no physical relationship to each other, but recent work has indicated the possibility that these two receptors might be different conformations of the same macromolecule (Kenakin et al., 1974). When strips of guinea pig ileum longitudinal muscle were exposed to temperatures below 20°C, the histamine receptor appeared to change from one possessing characteristics of an H_1 receptor to one with many properties of the H_2 receptor. It is thought that membrane lipids undergo a fluidity change at approximately this temperature, from a liquid to a more crystalline state, leading to the suggestion that the histamine receptor, which is believed to be located in the membrane, also undergoes a conformational change in conjunction with these lipids. This has not been confirmed in other histamine receptor systems, although a similar interchange of properties, from α-receptors to β-receptors and vice versa, has been detected in adrenergic receptors in heart (Kunos and Szentivanyi, 1968, Kunos and Nickerson, 1976).

Whether or not H_1 and H_2 receptors are two different conformational forms of the same macromolecule, it appears that events following the drug receptor interaction are not the same for each receptor sub-type. The H_2 receptor is believed to be associated with adenylate cyclase, since in cardiac muscle histamine-induced stimulation of H_2 receptors has been shown to be associated with an increase in cAMP (Klein and Levy, 1971; McNeill and Verma, 1974a). Burimamide, an H_2 receptor antagonist, effectively blocks both the mechanical effects of histamine and the increase in cAMP (McNeill and Verma, 1974a; Verma and McNeill, 1974; Reinhardt et al., 1974). On the other hand, stimulation of H_1 receptors in cardiac muscle, while also having an inotropic effect, is not associated with an increase in cAMP (Verma and McNeill, 1977).

4. Indirect Action of Histamine

Although histamine is believed to act in most tissues through a direct interaction at H_1 or H_2 receptors, in some instances histamine has been known to act indirectly through catecholamine release. The best known example of this is the release of catecholamines by histamine from the adrenal medullae of cats and dogs, which was first demonstrated by Burn and Dale (1926) and has often been demonstrated since (e.g., Feldberg, 1941; Staszewska-Barczak and Vane, 1965). The release of noradrenaline from nerve terminals by histamine, however, has only been demonstrated in a few instances, and in vascular smooth muscle no evidence for such an action of histamine has been found. In isolated chick ileum and cat tracheal chain histamine has been shown to have a dual mode of action. In cat tracheal chain preparations histamine causes relaxation; this appears to be mediated partly through H_1 receptors and partly through catecholamine release (Maengwyn-Davies, 1968). In chick ileum low doses of histamine appear to cause relaxation through catecholamine release, while higher doses produce a biphasic effect, the relaxation portion of which is mediated by catecholamine release, while the contraction portion is inhibited by H_1 antagonists

(Everett and Mann, 1967).

The release of noradrenaline by histamine or any other agent can be demonstrated by methods which either block the uptake of the releasing agent into the nerve terminal or reduce the total noradrenaline content of the nerve. These include the use of cocaine, which blocks neuronal uptake (Iverson, 1967), and reserpine, which depletes the nerve terminals of noradrenaline (Burn and Rand, 1958). In addition, destruction of sympathetic nerves can be achieved with 6-hydroxydopamine treatment or by chronic denervation (Burn and Tainter, 1931; Malmfors and Sachs, 1968).

III THE VENOUS SYSTEM

In this section, the properties of the portal veins of various species will be reviewed in detail, and what is known of actions of histamine in this tissue will be discussed.

A. General Information

Dr. William Harvey, who first described the cardiovascular system in 1628, recognized the existence of two major classes of vessels forming an integral part of the cardiovascular circuit. The function of these two classes of vessels is well known: one set, the arteries, takes oxygenated blood away from the heart to the tissues and the second set, the veins, then conduct the now deoxygenated blood from the tissues back to the heart. In general, arteries are considered to consist of resistance vessels, which are the main determinants of regional blood flow, while veins are considered to be capacitance vessels (Mellander and Johansson, 1968) which can contain up to 80% of the total blood volume in some species (Weidemann, 1963). Because of the large volume of blood located in the venous system at any one time, changes in the venous capacity can have a great influence on venous return and thereby on other variables in the circulatory system, such as blood pressure and regional blood volume (Mellander and Johansson, 1968). Changes in venous capacity can occur by two mechanisms: either indirect, through alterations in pre-capillary resistance, or directly through alterations in venous tone. Overall however, veins are not thought to play an important role in the active maintenance of circulatory homeostasis, for instance in response to neural or humoral stimuli. Yet for quite some time many veins have been known to respond both to electrical stimulation and to drugs (Franklin, 1937), and recently more and more research has been focused on the determination of the mechanisms controlling the activity of veins. The hepatic portal vein is an example of a vein which has recently been subjected to numerous pharmacological and electrophysiological studies, owing to its interesting characteristics which are reviewed below.

B. The Portal Vein

1. Location and Function

The portal vein, in man and other animals, is a large relatively short vessel formed by the junction of the splenic vein with the inferior and anterior (or superior in man) mesenteric veins. Other veins, such as the gastric and pyloric, also drain into it (Fig. 4). The portal vein travels from this junction to the liver where it divides into left and right branches, which on entering the liver bifurcate into radicles. These then drain into the liver sinusoids or capillaries (Hollinshead, 1974).

The hepatic portal vein, although carrying partially oxygenated blood, is not the only vessel to supply the liver - the hepatic artery also enters this organ. The mixture of blood from both these vessels then drains from the liver, via the hepatic veins, into the vena cava. While the hepatic artery carries normally oxygenated blood to the liver, it is apparent that some oxygen is also derived from the portal vein since blood in the hepatic veins has a lower O_2 content than that in the portal vein (Selkurt and Brecher, 1956). The relative amounts of oxygen supplied to the liver by each vessel varies widely depending on the hepatic blood flow and oxygen uptake, but a rough estimate is that at rest the portal vein accounts for about 65-75% of the blood supply, but only 30-35% of the O_2 requirement (Greenway and Stark, 1971).

As well as providing some of the liver oxygen supply, the portal vein also plays an important role in metabolism. It can be seen from the veins merging to form the portal vein that all blood from the digestive tract drains into the liver before it enters the general circulation. In this manner the concentration of substances which have been absorbed across the digestive tract and which are subject to biotransformation by liver enzymes is altered - the 'first-pass' effect. This is useful for the removal of substances which might be toxic when present in the general circulation and also has implications in the design of effective drug therapy. But though the portal vein is important for this function it is not essential for life - animals and man which have been subjected to either excision of the portal vein or to a porta-caval shunt, in which the portal vein is attached directly to the vena cava, by-passing the liver, are able to survive (Sedgwick and

Poulantzas, 1967).

2. Structure and Innervation

Blood vessels of all types have essentially the same composition, the components having been found to vary mostly in quantity, depending on the function and location of the vessel. The three major tissue components, which are arranged in concentric tubes are (Bevan and Su, 1973):

1) The intima: The innermost coat of the blood vessel, composed of a lining of endothelial cells, together with a supporting layer of connective tissue.
2) The media: The layer surrounding the intima, composed largely of vascular smooth muscle with elastic tissue. The smooth muscle is usually orientated in either a circular or a longitudinal direction, although some vessels contain two separate layers of smooth muscle, one of which is orientated in each direction.
3) The adventitia: The outermost layer of the blood vessel, coating the smooth muscle, is formed mainly from connective tissue.

Small venules and very large veins have been shown to be composed largely of intimal and adventitial layers, making them quite elastic in nature. Some of the intermediate sized veins, and many of the arteries, except the very large ones, also contain a well-developed medial layer.

The pattern of innervation of most blood vessels is also quite uniform, most differences between vessels consisting of variations in the density of innervation, associated with variations in the size and function of the vessels. Sympathetic nerves are usually found restricted to the perivascular plexus, which is an area of innervation located at the adventio-medial junction. In some large vessels nerves actually penetrate varying distances into the media layer from this plexus, but as a rule they make contact with relatively few smooth muscle cells. There are two means by which nervous impulses could be detected throughout the muscle under these circumstances: one is the diffusion of transmitter released from the nerve terminals throughout the tissue, and the second, thought to be more likely in the case of most vascular smooth muscle, is the propagation of smooth muscle electrical activity from cell to cell via nexuses (Dewey and Barr, 1962), areas of close apposition between the membranes of two cells (Ljung, 1970). Burnstock (1975) has made the suggestion that restriction of neurons to the advential side of the smooth muscle enables blood vessels to respond to dual control, by circulating catecholamines which can diffuse through the intima and most of the media without being removed by uptake into adrenergic nerve terminals, as well as by neuronal stimulation.

The structure and innervation of the portal vein has been examined in detail in both the rabbit and the rat, and these have been found to be very similar in both species. Electron microscopic examination of the rabbit portal vein (Holman et al., 1968) revealed that the medial layer of this tissue consists of two layers of smooth muscle cells, an inner circular layer about 30 μ wide, and an outer layer about 80 μ wide, arranged in longitudinal bundles which then form a very wide spiral. The same arrangement was detected in rat portal vein (Johansson et al., 1970; Funaki, 1967). In both species neighboring cells in the longitudinal smooth muscle layer could sometimes be seen to fuse to form tight junctions or nexuses. Further electron microscopic examination of these tissues, together with fluorescent histochemistry for the detection of catecholamines, indicated that two major areas of two-dimensional innervation could be found. One was the usual nerve plexus found at the adventio-medial border, and a second plexus was located between the longitudinal and circular muscle layers (Holman et al., 1968; Johansson et al., 1970; Burnstock et al., 1970). In the case of the rabbit, small bundles of axons were seen to penetrate into the media from the adventia via the spaces between the bundles of longitudinal smooth muscle. The minimum distance detected between membranes of these axons and those of smooth muscle cells was on the order of 1500 Å. Axons that were closer to, or more actually enveloped by, processes of smooth muscle cells were seen very rarely (Holman et al., 1968). The nerve plexus found in the area between the two muscle layers also consisted of bundles of axons, corresponding to noradrenergic nerves when examined with fluorescence microscopy. In the case of the rat portal vein almost the same picture was seen, although in this case the minimum neuromuscular distance found was about 1000 Å (Ljung, 1970). Ljung et al. (1973) performed studies on the uptake and distribution of ^{3}H-noradrenaline in rat portal vein, and based on their findings proposed that, at least in the rat, only the inner nerve plexus is functionally important for the portal vein. This nerve plexus seems to be a continuation of the adventia-medial nerve plexus of the anterior mesenteric vein which in the rat contains no longitudinal muscle. They also suggest that the outer nerve plexus may only follow the portal vein, not directly innervate it.

While fluorescence microscopy indicated that in both species much of the innervation of both nerve plexuses was sympathetic (Holman et al., 1968; Ljung, 1969), the actual proportion of the nervous supply that was noradrenergic in nature was then determined by pharmacological means.

3. Electrophysiological and Pharmacological Characteristics

a) Spontaneous Activity

The portal vein of every species examined so far has been shown to be spontaneously active both *in vivo* and *in vitro* (Hughes and Vane, 1967; Johansson and Ljung, 1967; Holman *et al*., 1968; Hall and O'Connor, 1973; Carruba *et al*., 1973). Spontaneous contractions of longitudinal strips of isolated rabbit portal vein were found to vary both in amplitude and in frequency, but in general they corresponded to a change of 10-15% of the total length of the muscle strip, and once the frequency was established, which often took up to an hour, the contractions were maintained for 6 to 8 hours (Hughes and Vane, 1967). Sutter studied the characteristics of what he termed rabbit anterior mesenteric-portal vein, which he described as corresponding to the portal vein of other reports. He found that only longitudinal strips were spontaneously active, ring preparations being quiescent even though he again demonstrated histologically the presence of circular smooth muscle in this preparation (Sutter, 1965). In general, the spontaneous activity of longitudinal and helical strips of portal vein isolated from other species was found to be somewhat greater in amplitude, relative to the length of the muscle, and similar in frequency to that found in the rabbit (Carruba *et al*., 1973; Johansson and Ljung, 1967a; Funaki, 1967). However, spontaneous activity detected *in situ* in the portal vein of the dog appeared to be a result of contributions from both longitudinal and circular smooth muscle (Hall and O'Connor, 1972). The spontaneous activity could be abolished by either lowering the temperature to 28-32°C in the rabbit, or to 34°C in the rat, or by decreasing the external calcium concentration (Holman _et_ _al_., 1968; Funaki, 1967; Cuthbert and Sutter, 1965; Axelsson _et_ _al_., 1967; Biamino and Johansson, 1970).

Electrical activity in the muscle was found to be associated with this spontaneous mechanical activity (Funaki and Bohr, 1964; Cuthbert and Sutter, 1964; Cuthbert _et_ _al_., 1965). Holman _et_ _al_. (1968) found electrical activity in the rabbit portal vein was very variable and could consist of single, pairs or bursts of action potentials. The activity associated with mechanical activity however consisted of waves of depolarization with bursts of spikes on their crests. They also found some evidence of pacemaker regions, where there was a steady fall in potential between the waves of depolarization, and each wave seemed to end in a hyperpolarizing trough from which the next depolarization developed. The same type of electrical activity was associated with the spontaneous mechanical activity in both rat and guinea pig portal vein, both of which also seem to possess pacemaker areas as well (Funaki and Bohr, 1964; Nakajima and Horn, 1967; Ljung and Stage, 1970).

The spontaneous activity of portal vein from the rat and rabbit is not affected by nerve-blocking concentrations of tetrodotoxin or cocaine, by hexamethonium, nor by dihydroergotamine, phentolamine, atropine or mepyramine (Sutter, 1965; Johansson and Ljung, 1967a; 1967b; Holman _et_ _al_., 1968). Chronic sympathetic denervation has no effect on the spontaneous activity (Johansson _et_ _al_., 1970). This evidence suggests that the spontaneous contractile activity of the muscle is myogenic in nature, arising from within the muscle and propagated throughout the tissue by electronic conduction. This in turn suggests that the smooth muscle of portal vein is of the 'single-unit' type described by Bolzer (1948) in which cell to cell propagation of electrical activity can occur, as opposed to multi-unit smooth muscle, where no synchronous activity is seen (Holman et al., 1968; Ljung, 1970). The presence of the nexuses, which as already mentioned, have been detected between some smooth muscle cells in the longitudinal coat, tends to support this view. Further support can be found from the work of Johansson and Ljung (1967b), who demonstrated that exposure of rat portal vein to hyperosmolar sucrose solutions resulted in a desynchronization of the spontaneous activity. This treatment had previously been shown to have no effect on conduction in autonomic nerves, but to disrupt cellular contacts at the nexuses in other types of smooth muscle (Barr et al., 1965a; 1965b).

b) Responses to Electrical Stimulation

When isolated longitudinal strips of portal vein from rats or rabbits were subjected to transmural stimulation both electrical and mechanical activity resulted. In general, the mechanical response at low values of stimulation consisted of an increased frequency of contraction which summed to produce an increase in tension. At higher values a steep rise in tension was found, followed by a more prolonged decay (Hughes and Vane, 1967; Holman et al., 1968; Johansson and Ljung, 1967a). The electrical response associated with threshold mechanical activity consisted of an increase in the frequency of bursts of spikes, corresponding to an increased frequency of contractions, suggesting according to Holman et al. (1968) either an increase in the slope of the pacemaker potentials or a decrease in the threshold for action potential initiation, or both. Electrical activity associated with increased contractile activity up to the maximum mechanical response, consisted of a depolarization of the membrane with continuous high frequency firing of action potentials (Holman _et_ _al_., 1968).

The mechanical and electrical activity resulting from transmural stimulation appeared to be caused by excitation of nervous tissue, since it could be prevented with chronic denervation (Johansson _et_ _al_., 1970) and could be blocked with guanethedine, and selective nerve-blocking concentrations of tetrodotoxin (Holman _et_ _al_., 1968), cocaine and lignocaine (Hughes and Vane, 1967). Veratrine, an agent which causes repetitive nerve firing, increased the effects of transmural stimulation (Hughes and Vane, 1967).

The transmitter released after nerve stimulation of rabbit portal vein was identified as noradrenaline, both chemically and pharmacologically (Hughes, 1972a). The excitatory response to nerve stimulation could be blocked with phentolamine, an α-adrenergic antagonist, as well as the adrenergic neuron blockers bretylium and bethanidine. In addition, the contractile response to electrical stimulation was abolished in tissues taken from rabbits pretreated with reserpine (Holman _et_ _al_., 1968; Hughes and Vane, 1967). On the other hand, the response to electrical stimulation was found to be unaffected by hyoscine, mepyramine or brom-lysergic acid diethylamine (brom-LSD), or by the ganglion blocking agents hexamethonium or mecamylamine (Hughes and Vane, 1967). It thus appears that the contractile response of rabbit portal vein to nerve stimulation is mediated via noradrenaline acting at α-adrenergic receptors.

Isolated strips of portal vein taken from rabbits pre-treated with reserpine show relaxation with electrical stimulation (Hughes and Vane, 1967). In addition, tissues treated with high concentrations of phentolamine also showed relaxation when stimulated (Holman *et al.*, 1968; Hughes and Vane, 1967). In both cases, Hughes and Vane (1967) found that the inhibitory effect could be only partially antagonized with propanolol and INPEA, which are β-adrenergic antagonists. The remainder of the inhibitory effect, resistant to both α- and β-receptor blockade, was shown to be of neuronal origin, since it was reduced by lignocaine and cocaine and increased by veratrine, as well as being abolished by tetrodotoxin in selective nerve-blocking concentrations. However they were unable to block this inhibitory effect with any of the common receptor antagonists, including antagonists of the acetylcholine, 5-hydroxytryptamine or histamine responses.

The results reviewed above indicate that the major innervation of the rabbit portal vein is adrenergic in nature, confirming the histochemical studies. This has been further confirmed by stimulation of the splanchnic and vagus nerves to the portal vein, both in the cat (*in situ*) and in the rabbit (*in vitro*). Stimulation of both right and left splanchnic nerves resulted in active contraction of the portal vein, which could be blocked by guanethidine, phenoxybenzamine and phentolamine, and further stimulation after blockade then had no effect. Stimulation of the vagus nerve had no mechanical effect on the portal vein (Johansson and Ljung, 1967b).

These results provide further indication that the major sympathetic control is exerted via excitatory α-adrenergic receptors, while there also appears to be β-adrenergic receptors which are inhibitory, located in the muscle. Another non-adrenergic inhibitory innervation may also be present but the nature of the transmitter is unknown, and it does not appear to be present in portal veins of other species (Ljung, 1970; Funaki, 1964).

c) Storage, Release, Distribution and Inactivation of Noradrenaline

Noradrenaline, the sympathetic neurotransmitter in both rat and rabbit portal vein, is stored in adrenergic nerve terminals which were shown histochemically to be restricted to the adventitio-medial border, penetrating somewhat in the spaces between longitudinal muscle bundles, and to the junction between the longitudinal and circular smooth muscle layers (Holman *et al*., 1968; Johansson *et al*., 1967). Noradrenaline appears to be stored in different pools within the nerve terminal, and can be released on nerve stimulation, the pool from which it arises varying depending on the stimulation parameters (Hughes and Roth, 1974). Noradrenaline released at low frequencies of stimulation, for instance, appears to be from a newly synthesized source rather than from that stored for longer periods (Greenberg, 1975). There is some evidence that in rabbit portal vein, noradrenaline release is subject to modulation by prostaglandins (Greenberg, 1974). Treatment of the isolated vein with indomethacin, a prostaglandin synthetase inhibitor, results in an increased output of noradrenaline at low frequencies of stimulation, while treatment with prostaglandin E_2 decreases the noradrenaline released on electrical stimulation. These results suggest that prostaglandins may have a regulatory role in noradrenaline release in this tissue, as it does in others.

The noradrenaline that is released on nerve stimulation is released into a restricted area, on the outer edge of the smooth muscle layers. In the case of rat portal vein, evidence exists to suggest that the functionally important α-adrenergic receptors are restricted in distribution as well. Based on the supersensitivity exhibited by rat portal vein after chronic denervation and cocaine treatment, Ljung _et al_. (1973) suggested that α-receptors are located near the nerve plexus, and are probably confined to 'innervated' muscle cells, located close to the nerve terminals. The response of these muscle cells to noradrenaline is then probably propagated electrically throughout the tissue, by myogenic conduction, in the same manner as the spontaneous activity. Bevan and Ljung (1974) have shown that when rat portal vein is stimulated 'locally' it responds with almost as great a change in length as when it is subjected to transmural (general) field stimulation.

Noradrenaline action is terminated by a combination of neuronal and extraneuronal uptake (Hughes, 1972a). In normal tissues, a balance seems to exist between the two processes, which together appear to inactivate over 90% of the released transmitter, since if one uptake process is blocked then the other process increases in relative importance. This is supported by the fact that noradrenaline is subject to breakdown by both metabolizing enzymes, catechol-O-methyl transferase, an extraneuronal enzyme, and monoamine oxidase, located in the nerve terminal.

d) Responses to Drugs

As would be expected from the preceding discussion, the addition of exogenous noradrenaline mimics almost exactly the action of nerve stimulation, both electrically and mechanically. The major difference appears to be that responses to added noradrenaline were slower in onset (Holman _et al_., 1968; Johansson _et al_., 1967). Adrenaline action was similar to that of noradrenaline, and the contractile responses to both these antagonists could be abolished by the α-adrenergic antagonist, phentolamine. In the presence of high concentrations of phentolamine, large doses of noradrenaline and adrenaline caused an inhibitory response, blocked by propanolol, as also occurred with electrical stimulation (Holman et al., 1968; Hughes and Vane, 1967).

Isoprenaline, the β-adrenergic agonist, was also found to cause inhibitory responses, which could be blocked with propanolol (Holman et al., 1968; Johansson et al., 1967; Hughes and Vane, 1967; Cohen and Wiley, 1977).

In contrast to the relatively high sensitivity exhibited by the rat and rabbit portal veins to the adrenergic agonists, the portal veins of these and other species were found to be considerably less sensitive to acetylcholine, histamine and 5-hydroxytryptamine. The responses to these agonists have been examined in rat, guinea pig, rabbit and dog portal veins, and in general there appears to be little species variation, although the effective concentrations seemed to vary somewhat. All of these tissues responded to acetylcholine, and the response to this agonist could be blocked by atropine but not by hexamethonium (Carruba et al., 1973; Hughes and Vane, 1967; Sutter, 1965). 5-Hydroxytryptamine also elicited contractions of the portal veins of each species examined, and the responses to this agonist could be blocked with Brom-LSD and methysergide (Hughes and Vane, 1967; Sutter, 1965), indicating an action of 5-HT on so-called 'D' receptors of smooth muscle (Gaddum and Picarelli, 1957). In further support of this conclusion, the action of 5-HT was not affected by atropine (Hall and O'Connor, 1973). Hughes and Vane (1967) reported the ability of phentolamine, the α-adrenergic antagonist, to inhibit the response of rabbit portal vein to 5-HT, in approximately the same concentrations as required to block the responses to noradrenaline and adrenaline, although they also found brom-LSD was an effective antagonist of the 5-HT response.

The portal veins of all these species were found to respond with varying degrees of sensitivity to histamine, which also caused contractions. Although Carruba *et al*. (1973) found that the response of rabbit portal vein to histamine was insignificant, other reports indicated that this tissue could, in fact, respond to this compound, albeit quite weakly (Hughes and Vane, 1967; Sutter, 1965). The response to histamine was considered to be mediated by H_1 receptors in both the rabbit and dog portal vein, since the response could be blocked with mepyramine and antazoline but not with metiamide, an H_2 antagonist (Hughes and Vane, 1967; Sutter, 1967; Thermann *et al*., 1975; Richardson and Withrington, 1977b).

In addition to the actions of the aforementioned compounds, the portal veins of various species have been reported to respond to a number of other vasoactive compounds. Angiotensin causes contraction, with subsequent tachyphylaxis, of dog, rabbit and rat portal vein. Bradykinin has been reported to cause contraction of rat and guinea pig portal vein, but not preparations from rabbit or dog. On the other hand, oxytocin and vasopressin have been found to produce relaxation of portal vein from all species examined, and in addition rabbit portal vein has been reported to be inhibited by ATP, ADP and AMP (Hughes and Vane, 1967; Carruba *et al*., 1973; Richardson and Withrington, 1977a).

IV GENERAL REMARKS

It is clear from the preceding section that the portal vein is relatively well-innervated, and responds both to nerve stimulation and to exogenous drugs. The function of the portal vein in the control of blood pressure and blood volume in the systemic circulation, although not well-understood, is thus potentially very important. Studies on whole animals indicated that in both the cat and the dog, stimulation of the splanchnic nerve to the intestine results in an increase in portal venous pressure, and an initial increase, followed by a decrease in portal flow (Green et al., 1959; Johansson and Ljung, 1967a). The increase in portal pressure and portal resistance seems to be a direct result of nerve stimulation on the smooth muscle of the portal vein, but this in itself had little effect on portal flow. The changes in portal flow could be related to changes in the volume of the intestinal capacitance vessels. In view of these results, Greenway and Stark (1971) suggested the possibility that in response to a decrease in splenic or intestinal blood flow, the constrictory response of portal vein to reflex sympathetic stimulation would minimize any fall in portal pressure and could aid in the maintenance of the intestinal capillary resistance, and in the prevention of the collapse of the intestinal blood vessels.

The results reviewed previously also indicated that while the portal veins of various species responded to 5-HT and histamine, these agents were less effective at causing portal constriction. In fact, it has been demonstrated that in the dog, infusion of relatively low doses of histamine results in an increase in portal venous pressure, most of which seems to be due to an outflow block from the liver due to constriction of the hepatic veins (Richardson and Withrington, 1977b; Greenway and Oshiro, 1973). Under normal circumstances neither histamine nor 5-HT are likely to be present in vasoactive amounts, but both substances can be released into the circulation under conditions of anaphylaxis or other stimuli, and under these circumstances the blood levels of these substances are likely to become high enough to cause constriction of the portal vein directly.

As mentioned previously, the aim of the research reported in the following chapters was to examine in more detail the actions of histamine in the isolated portal vein, since the effects of histamine on venous smooth muscle are not well known.

METHODS

I CONTRACTILITY STUDIES

New Zealand white rabbits, weighing 1.5-3.0 kg, were killed by cervical dislocation. The hepatic portal vein was immediately disected free from the surrounding mesentery, excised, and placed in warm oxygenated Krebs solution. The vessel was cleaned of as much remaining fat and connective tissue as possible, before being cut into a wide spiral strip, approximately 1 cm wide and 3 cm long. The spiral strip was divided into either 4 or 6 equivalent pieces along its longitudinal axis. Each piece was suspended under 5 gm tension in an isolated tissue bath with a working volume of either 15 ml or 28 ml. The baths contained Krebs solution (see Appendix for composition), maintained at 37°C and bubbled with 95% oxygen - 5% CO_2. Contractions of the tissues were measured isotonically, using Hewlett-Packard 7DCDT linear motion transducers, and recorded on a Grass Model 5PI polygraph.

Tissues were allowed to equilibrate for one hour in normal Krebs solution during which time they were washed every 20-30 minutes. After 60 minutes the sensitivity of the tissues was determined either to a maximum dose of agonist or to 10^{-1}M potassium chloride before the experiment was begun.

Dose-response curves to all agonists were obtained in a cumulative manner, graded doses being added to give the desired concentration of drug in the bath. A constant bath volume was maintained by means of a vacuum apparatus which removed any fluid overflow. All drugs were prepared and diluted in Krebs solution in order to prevent any dilution of that solution in the bath.

In all experiments, except those involving the use of phenoxybenzamine, when more than one dose response curve was obtained on a single tissue, the tissue was washed for 60 minutes between each curve to avoid any complications due to desensitization. When reversible antagonists were used, this wash time was suitably adjusted for the onset and offset of antagonism. Often more than one concentration of competitive antagonist was added to a tissue. Under these circumstances, a control dose-response curve to agonist alone was obtained before the second dose of antagonist was added, in order to ensure that complete recovery from blockade had taken place.

A. Reserpinized Tissues

Portal veins were obtained from animals which had been pre-treated with 2.0 mg/kg of reserpine subcutaneously 16 hours, and 1.0 mg/kg of reserpine intravenously 1 hour before being killed (Paton, 1973).

B. Treatment with 6-hydroxydopamine (6-OHDA)

After the equilibration period the responses of strips of portal vein were tested to maximum doses of various agonists. Then, in a modification of the *in vitro* technique of Aprigliano *et al.* (1976), 250 μg/ml of 6-OHDA was added to each bath, together with 1.1 mg ascorbic acid to reduce auto-oxidation of this compound (Wadsworth, 1973). The 6-OHDA was left in contact with the tissue for 15 minutes, and then the tissues were washed with normal Krebs solution for 2 hours, or until a stable response was obtained. Responses to agonists were again obtained, and these results were expressed as a percentage of the initial response.

C. Electrical Stimulation

In some instances it was necessary to measure the response of strips of portal vein to transmural electrical stimulation. Tissues were threaded between two circular platinum electrodes mounted on a perspex cylinder before being attached to the transducers, and were stimulated using a Grass SD9 stimulator. Tissues were stimulated with square wave pulses at maximum voltage output (100 volts), with a frequency of 25 Hz, and a pulse width of 2 msec.

D. Induction of Tachyphylaxis or Desensitization

Tachyphylaxis and desensitization are both terms which refer to the process by which a dose of a drug produces a smaller response to a subsequent dose. Although the same procedure was followed to induce both of these phenomena, the molecular explanation of tachyphylaxis is different from that of desensitization. **Tachyphylaxis** in this discussion, refers to the reduction in the response of a tissue to an indirectly-acting agent and is thought to be caused by the depletion of the neurotransmitter released by this agent (Axelrod *et al*., 1962). Desensitization, on the other hand, refers to the reduction in the response to a directly-acting agent, which, when responses to other agonists are not reduced, is believed to result from an alteration in the receptor (Rang and Ritter, 1970). In each case, a control response to each agonist was obtained. The tissues were then exposed to repeated doses of the desensitizing agent for varying lengths of time, until the contractile response was reduced to less than 50% of the initial response to that agent. Between each exposure the tissues were washed repeatedly, until the response returned to **baseline**, before the next dose was added. When the response to the desensitizing agent was sufficiently reduced, a second response was obtained to each test agonist. These results were then expressed as a percentage of the initial response to that agonist.

E. Blockade with Phenoxybenzamine

Phenoxybenzamine (POB) was dissolved in 0.01 N hydrochloric acid and stored at room temperature for 20 minutes. POB, as with other β-haloalkylamines, is believed to act as an irreversible antagonist via the formation of a cyclic aziridinium ion, which forms covalent bonds with nucleophilic sites located on the cell (Fig. 5) (Harvey and Nickerson, 1953; Graham, 1957). Previous studies have shown that optimal cyclization of the POB molecule to form the aziridinium ion takes place within 15-40 minutes (Kenakin and Cook, 1975). After 20 minutes the solution of antagonist was then diluted to the required concentration in normal Krebs solution and used immediately.

The experimental procedure followed is outlined in Fig. 6. Initially a control dose-response was obtained on all four tissues, after which they were washed for 15 minutes. Phenoxybenzamine was added to two tissues, left in contact with them for 3 minutes, and the tissues were then washed. The other two tissues were retained as unblocked, untreated controls. All four tissues were then washed for 15 minutes with Krebs solution containing sodium thiosulfate (10^{-3}M). Finally, a second dose-response curve was obtained.

The purpose of including sodium thiosulfate in the wash was to aid in the removal of any untreated aziridinium ion. The thiosulfate ion has been known for years to react very quickly *in vitro* with aziridinium ion, resulting in the formation of a pharmacologically inert compound known as a Bünte salt (Fig. 7) (Fruton *et al*., 1946). Prior administration of thiosulfate to preparations treated with β-haloalkylamines resulted in a great reduction or abolition of the action of these antagonists, indicating that their blocking activity does, in fact, arise from formation of the aziridinium ion (Nickerson and Gump, 1949; Graham and Lewis, 1954).

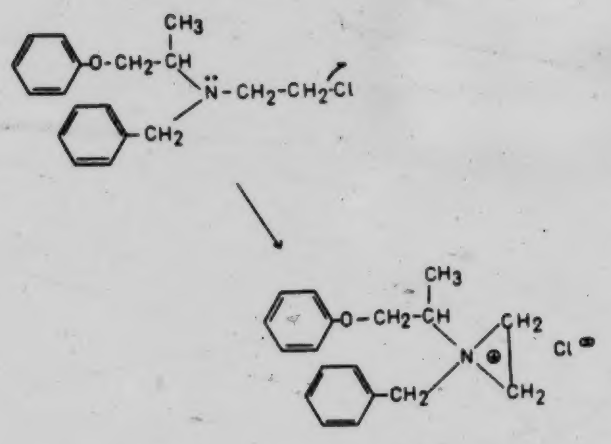


FIG. 5 The formation of the aziridinium ion from phenoxybenzamine.

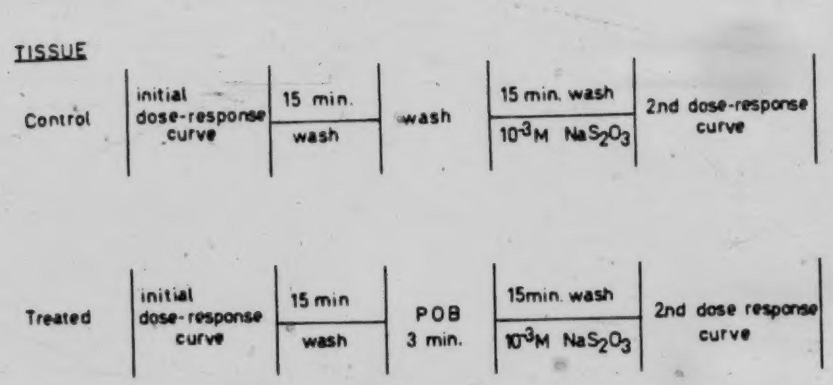

TISSUE
Control
initial dose-response curve
15 min. wash
wash
15 min. wash
10^{-3}M NaS_2O_3
2nd dose-response curve
Treated
initial dose-response curve
15 min wash
POB 3 min.
15min. wash
10^{-3}M NaS_2O_3
2nd dose response curve

FIG. 6 Schematic diagram illustrating the procedure followed in experiments utilizing phenoxybenzamine (See Results I-E).

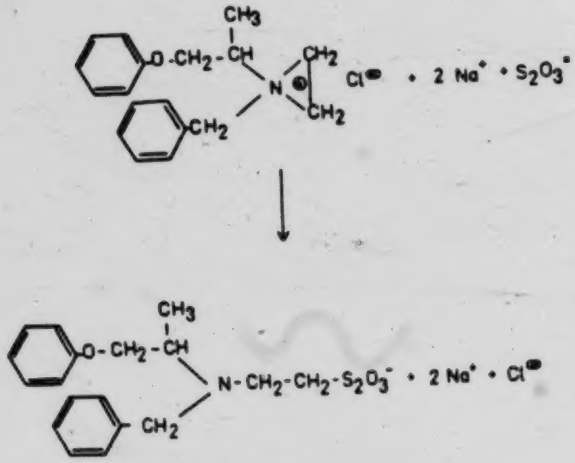

CH3
O-CH2-CH
CH2
N ⊕
CH2
CH2
Cl⊖ + 2 Na+ + S2O3=
CH3
O-CH2-CH
N-CH2-CH2-S2O3- + 2 Na+ + Cl⊖
CH2

FIG. 7 The reaction of aziridinium ion and sodium thiosulfate to form an inactive Bünte Salt.

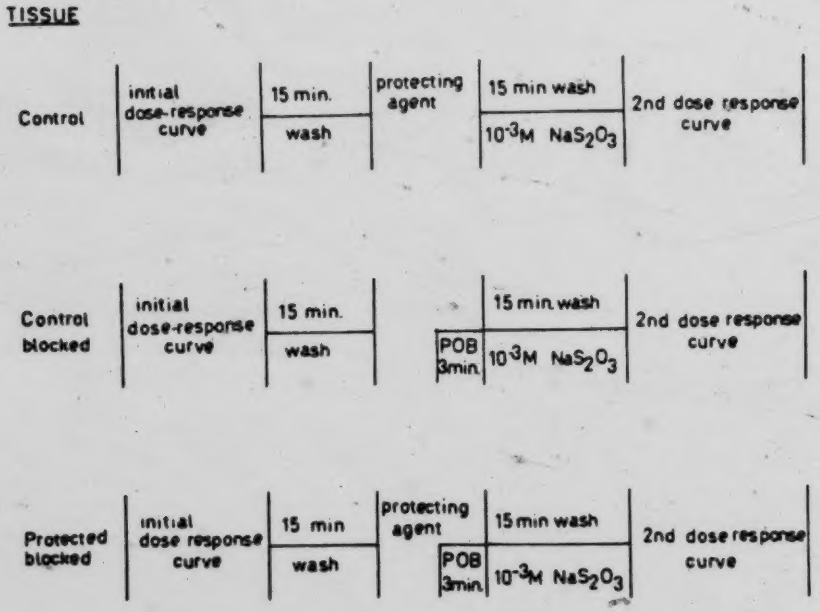

TISSUE
Control
initial dose-response curve
15 min.
wash
protecting agent
15 min wash
10-3M NaS2O3
2nd dose response curve
Control blocked
initial dose-response curve
15 min.
wash
POB 3min.
15 min wash
10-3M NaS2O3
2nd dose response curve
Protected blocked
initial dose response curve
15 min
wash
protecting agent
POB 3min.
15 min wash
10-3M NaS2O3
2nd dose response curve

FIG. 8 Schematic diagram illustrating the procedure followed in receptor self- and cross-protection experiments. (See Results I-F).

F. Receptor Protection

The experimental procedure used in receptor self-protection and cross-protection is outlined in Fig. 8. When the responses to histamine and 5-hydroxytryptamine were to be protected, it was necessary in most cases to use 6 tissues, divided into 3 pairs. One pair was treated with phenoxybenzamine alone ('control-blocked'), one pair treated with protecting agent alone ('control'), and one pair treated with both protecting agent and POB ('protected-blocked'). In this way it was possible to monitor both the sensitization of the tissue to histamine and 5-HT, while at the same time to have a measure of the effects of the protecting agent alone on the response to these agonists. The acetylcholine protection experiments were also performed in this manner, but this procedure was not required for the noradrenaline protection experiments: responses to this agonist do not show sensitization (see Results), and they are remarkably resistant to alteration by the protecting agent alone.

An initial dose-response curve was obtained on all tissues, after which they were washed for 15 minutes. The tissues designated 'control' and 'protected-blocked' were treated with protecting agent, and after sufficient time had elapsed for the response to become stable, POB was added to the appropriate tissues in the presence of protecting agent. After a 3 minute exposure to POB, all tissues were washed with Krebs solution containing sodium thiosulfate (10^{-3}M), for either 15 minutes, or when phentolamine, diphenhydramine or noradrenaline were used as protecting agents, 50 minutes. Then a second dose-response curve was obtained on each tissue.

G. Calculation and Expression of Results

Dose-response curves were constructed in most cases by taking the maximum response of the initial dose-response curve as 100% and calculating all remaining dose-response curves as a percentage of this value. In the case of experiments in which the histamine response was blocked with more than one dose of competitive antagonist however, the final dose of antagonist was not added until at least 4 hours after the initial dose-response curve was obtained. Since the histamine response sensitizes with time, in order to both minimize the error due to sensitization and to simplify the expression of results, both dose-response curves obtained in the presence of antagonist were calculated as a percentage of the maximum response of the normal dose-response curve obtained between them.

Results, when necessary, were analyzed for significance using the Students paired or unpaired t-tests.

II STUDIES WITH [^{3}H]-NORADRENALINE

Portal veins were prepared as described in Part I for contractility experiments, up to the point where the vein was cut into a wide spiral strip. In these experiments the spiral strip was divided into 8 equivalent pieces along its longitudinal axis, and these were mounted on fine hollow tissue hooks, attached to lines supplied with 95% O_2 - 5% CO_2 and placed in Krebs solution maintained at 37°C.

Tissues were prepared and labelled according to the method of Paton (1973). All Krebs solution contained both 1.1 x 10^{-4}M ascorbic acid (20 mg/l) and, except where specifically indicated, tropolone, (10^{-4}M), a catechol-O-methyl transferase inhibitor (Belleau and Burba, 1963).

Tissues were allowed to equilibrate in normal Krebs solution for 15 minutes, after which they were usually incubated for 30 minutes in Krebs solution containing pargyline (5×10^{-4}M), a monoamine oxidase inhibitor (Zeller, 1966). They were then transferred into fresh Krebs solution for a further 15 minutes, before being subjected to one of the experimental procedures described below.

DL-[7-^{3}H]-noradrenaline (Fig. 9), of specific activity 14.8 Ci/mmol, was obtained from Amersham/Searle Corporation. It was stored in the dark at 5°C and was diluted to the required concentration immediately before use.

Tissues were weighed when necessary on a Cahn Electrobalance (Model G) or a Cave AE balance; tissue wet weights were in the range of 3-8 mg.

A. Sample Preparation

The preparation of samples for counting was the same regardless of the nature of the experiment. In the case of samples of medium, a 0.5 ml aliquot of each solution to be counted was placed in a 7 ml polyethylene mini-vial, to which 5 ml of Aquasol® (New England Nuclear), a xylene-based scintillation cocktail, was also added. Vials were dark-adapted overnight (a minimum of 18 hours) and the following day were counted in a Beckman LS 230 scintillation counter. Dpm (disintegrations per minute) of each sample of medium was determined by the equation (1):

$$(1) \quad \text{Dpm} = \frac{\text{Cpm}}{\text{Efficiency}} \times \text{aliquot factor} \quad \text{(Cook, 1975)}$$

The counting efficiency was determined by the internal standard channels

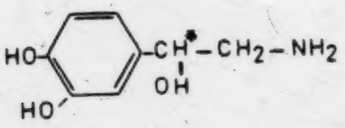

HO
HO
CH*
CH2
NH2
OH

FIG. 9 The chemical structure of noradrenaline. * indicates the position at which the greatest amount of tritium is bound.

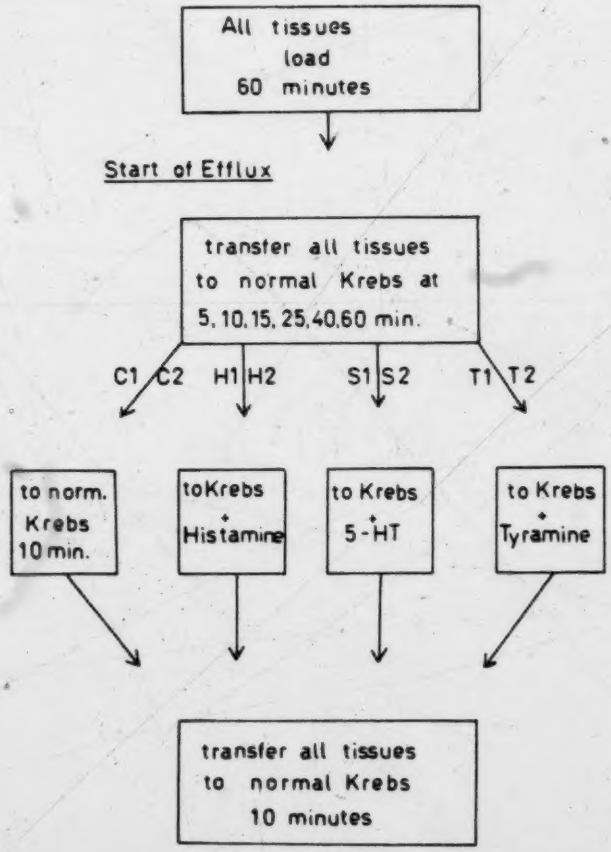

All tissues load 60 minutes
Start of Efflux
transfer all tissues to normal Krebs at 5, 10, 15, 25, 40, 60 min.
C1 C2
H1 H2
S1 S2
T1 T2
to norm. Krebs 10 min.
to Krebs + Histamine
to Krebs + 5-HT
to Krebs + Tyramine
transfer all tissues to normal Krebs 10 minutes

FIG. 10 Flow chart illustrating the procedure followed in experiments monitoring the efflux of [^{3}H]-noradrenaline under control conditions, and in the absence of MAO and COMT inhibitors. (See Results II-E).

ratio technique, described in the next section.

Tissue samples were blotted, weighed when necessary and placed in a mini-vial. 0.2 ml of NCS solubilizer (a quaternary ammonium base mixture in toluene) was added to each vial, and the vials were placed in a water bath maintained at 50°C overnight. The following day, 0.5 µl glacial acetic acid was added to each vial to neutralize the NCS and then 5 ml of a toluene base scintillation fluor (see Appendix for composition) was also added. Samples were dark-adapted for a minimum of 30 minutes before being counted. Tissue disintegrations per minute were determined from equation (2):

$$\text{(2)} \qquad \text{Dpm} = \frac{\text{Cpm}}{\text{Efficiency}}$$

B. Determination of Counting Efficiencies

Counting efficiencies were determined separately for medium and tissue samples by the internal standard channels ratio technique.

A 10 µl aliquot of the stock solution of [^{3}H]-noradrenaline was diluted in 1 ml of Krebs solution. Aliquots of this diluted stock solution (containing 2.2 x 10^{-7} dpm) were then added to vials containing either unlabelled medium (0.5 ml) or tissue which had been previously solubilized with NCS and neutralized with glacial acetic acid. After addition of the appropriate scintillation fluor, both medium and tissue standards were counted in two channels of pre-set window width. A channel ratio was calculated for each standard from equation (3):

$$\text{(3)} \qquad R = \frac{\text{Cpm in window B (narrow)}}{\text{Cpm in window A (wide)}}$$

The counting efficiency was also determined for each standard by use of equation (4):

$$(4) \quad \text{Percent (\%) Efficiency} = \frac{\text{Cpm in window A}}{\text{Total dpm added}} \times 100$$

The channels ratio was plotted against the counting efficiency separately for tissue standards and medium standards. The medium standards were all found to have a channels ratio within a narrow range, and counting efficiencies between 28% and 36%, so all medium samples whose R values also fell in this range were corrected by a factor of 32%. The graph of the tissue standards channels ratio versus counting efficiency was fitted with a straight line by the least squares method. A computer programme was then devised to derive the counting efficiencies of tissue samples, by comparison of their channels ratios to the straight line of the standard curve.

Background counts were determined for all experiments by counting appropriate blanks. These counts rarely rose above 50 cpm and were thus ignored.

C. Uptake of [^{3}H]-Noradrenaline

Sixteen tissues were prepared and divided into four groups of four tissues each. Each group of four tissues was transferred to a 25 ml Erlenmeyer flask. Two of these flasks were designated 'controls', and contained only 3 x 10^{-7}M [^{3}H]-noradrenaline in 5 ml of Krebs solution. The other two flasks were designated 'cocaine-treated' and contained as well as 3 x 10^{-7}M [^{3}H]-noradrenaline, 3 x 10^{-5}M cocaine in 5 ml of Krebs solution. Tissues were removed from each flask at exactly 3 minutes, 10 minutes, 30 minutes and one hour after the start of the experiment. Tissues were blotted, weighed, and then prepared and counted as described above.

D. Calculation and Expression of Results

Tissue sample counts were corrected for efficiency and used to determine the dpm per mg of tissue. Then the tissue : medium ratio was determined for each tissue. The T/M ratio is defined by equation (5):

$$(5) \qquad T/M = \frac{\text{Dpm/mg tissue}}{\text{Dpm/ml media}}$$

The T/M ratios for each sample from each group at each time were pooled and plotted against time to obtain a curve reflecting [^{3}H]-noradrenaline uptake.

E. Efflux of [^{3}H]-Noradrenaline

In experiments in which the efflux of [^{3}H]-noradrenaline was monitored, tissues were loaded with noradrenaline for one hour from a solution containing 3 x 10^{-7}M [^{3}H]-noradrenaline. Then efflux was followed under one of the following sets of conditions:

(1) Control conditions.

(2) In the absence of monoamine oxidase and catechol-O-methyl transferase inhibitors.

(3) In the presence of cocaine.

(4) In the presence of 6-hydroxydopamine.

The procedure followed in the control experiments is outlined in Fig. 10. After loading, each tissue was transferred to a tube containing 1.0 ml of normal Krebs solution. Each tissue was transferred again, at 5 minutes, 10 minutes, 15 minutes, 25 minutes, 40 minutes and 60 minutes, each time to a tube containing 1.0 ml of normal Krebs solutions. At 70 minutes each tissue was transferred again, two into normal Krebs, two into Krebs solution containing 5 x 10^{-3}M histamine, two into Krebs solution containing 5 x 10^{-4}M 5-hydroxytryptamine and the last two into Krebs containing 10^{-4}M tyramine. After a 10 minute exposure to drug, all 8 tissues were transferred to normal Krebs for a further 10 minutes. A 0.5 ml aliquot from each tube at each time interval was then counted, as were all tissues.

In the second set of experiments, the tissues were not exposed to pargyline before loading, and tropolone was omitted from the Krebs solution. Otherwise the experiments were run exactly as those described above (Fig. 10).

In experiments involving exposure to cocaine or 6-hydroxydopamine, tissues were grouped as shown in Table 1, first into 2 sets of four, then into pairs, one from each set.

Experiments using cocaine were very similar to the control experiments described above. The procedure followed is outlined in Fig. 11. All eight tissues were treated as described for the control preparations until 60 minutes of efflux had taken place. Then the four tissues designated as 'cocaine-treated' were transferred to Krebs solution containing 3 x 10^{-5}M cocaine for 10 minutes, while the control tissues were put in normal Krebs solution. At 70 minutes all eight tissues were transferred to the appropriate drug solutions as indicated in Table 1, the 'cocaine-treated' tissues still in the presence of cocaine. At 80 minutes all eight tissues were placed in normal Krebs for a final 10 minutes. Again a 0.5 ml aliquot from each tube at each time interval, and all tissues were counted.

The procedure involving 6-OHDA varied somewhat from the preceding experiments, and is shown in Fig. 12. After loading, the 4 tissues designated as 6-OHDA treated were transferred to a tube containing 250 µg/ml of 6-OHDA for 15 minutes, while the control tissues were

TABLE I

Nature of treatment of tissues in [^{3}H]-noradrenaline efflux experiments involving cocaine and 6-OHDA.

Cocaine		6-OHDA	
Tissue	Treatment	Tissue	Treatment
C1	untreated	C1	untreated
C2	cocaine	C2	6-OHDA
H1	histamine	H1	histamine
H2	cocaine + histamine	H2	6-OHDA + histamine
S1	5-HT	S1	5-HT
S2	cocaine + 5-HT	S2	6-OHDA + 5-HT
T1	tyramine	T1	tyramine
T2	cocaine + tyramine	T2	6-OHDA + tyramine

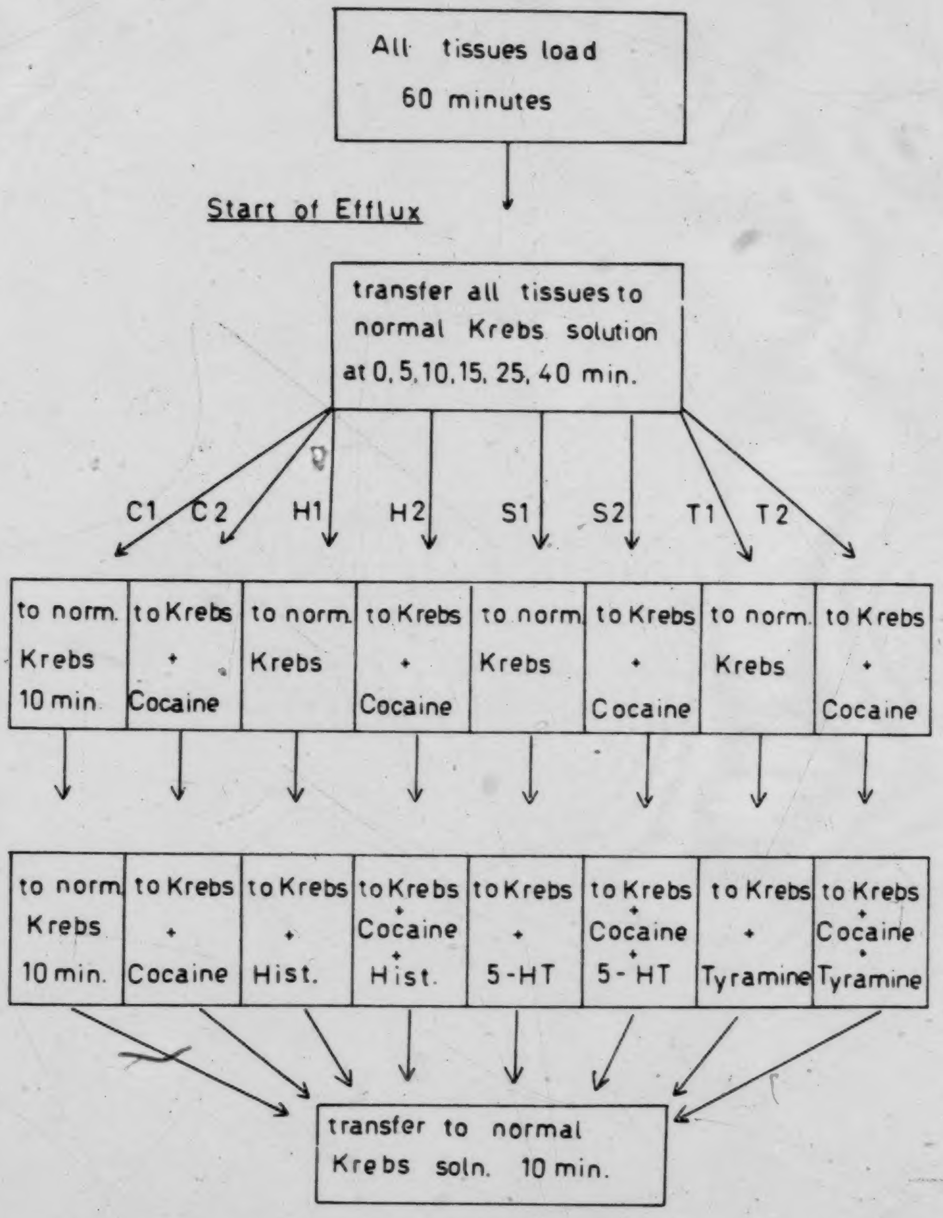

All tissues load
60 minutes
Start of Efflux
transfer all tissues to
normal Krebs solution
at 0, 5, 10, 15, 25, 40 min.
C1
C2
H1
H2
S1
S2
T1
T2
to norm. Krebs 10 min
to Krebs + Cocaine
to norm. Krebs
to Krebs + Cocaine
to norm. Krebs
to Krebs + Cocaine
to norm. Krebs
to Krebs + Cocaine
to norm. Krebs 10 min.
to Krebs + Cocaine
to Krebs + Hist.
to Krebs + Cocaine + Hist.
to Krebs + 5-HT
to Krebs + Cocaine + 5-HT
to Krebs + Tyramine
to Krebs + Cocaine + Tyramine
transfer to normal
Krebs soln. 10 min.

FIG. 11 **Flow chart illustrating the procedure followed in experiments monitoring the efflux of [^3H]-noradrenaline in the presence and absence of cocaine. (See Results II-E).**

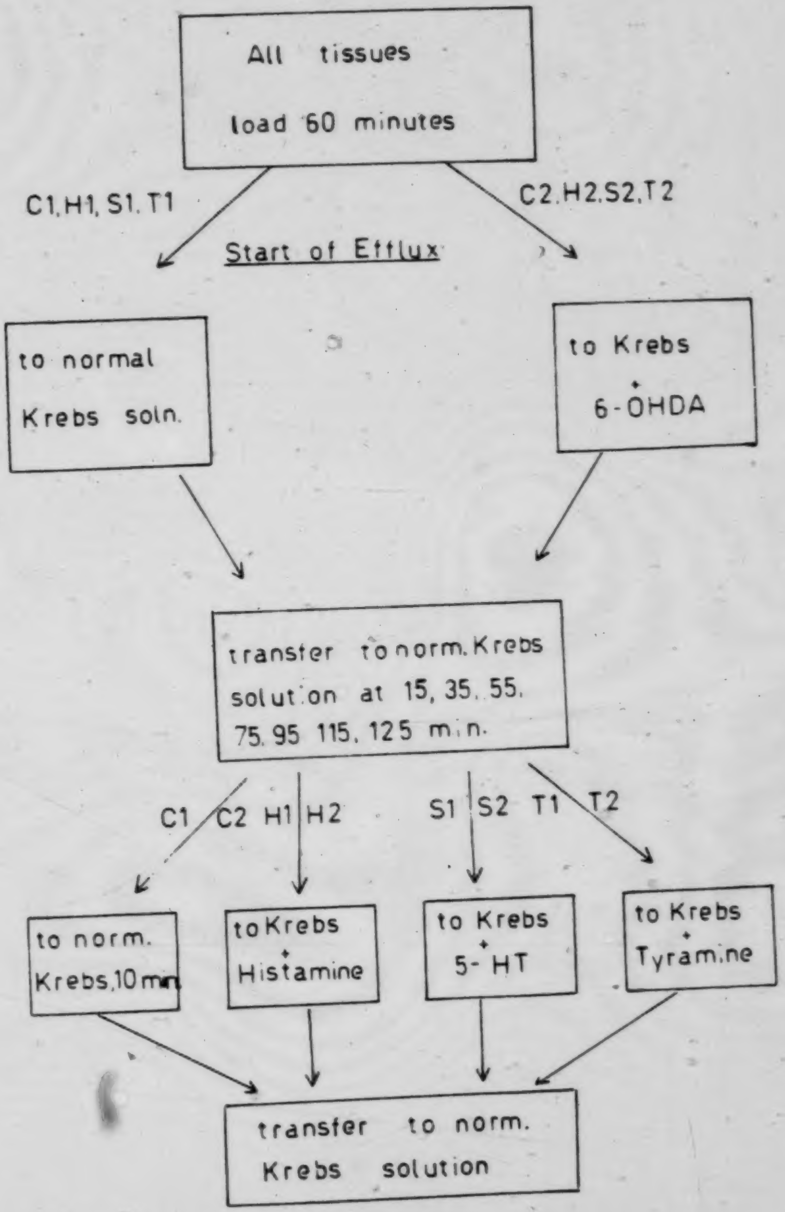

All tissues
load 60 minutes
C1, H1, S1, T1
C2, H2, S2, T2
Start of Efflux
to normal
Krebs soln.
to Krebs
+
6-OHDA
transfer to norm. Krebs
solution at 15, 35, 55,
75, 95 115, 125 min.
C1
C2
H1
H2
S1
S2
T1
T2
to norm.
Krebs, 10 min
to Krebs
+
Histamine
to Krebs
+
5-HT
to Krebs
+
Tyramine
transfer to norm.
Krebs solution

FIG. 12 Flow chart illustrating the procedure followed in experiments monitoring the efflux of [^{3}H]-noradrenaline from tissues treated with 6-OHDA. (See Results II-E).

placed in normal Krebs during this time. All tissues were then placed in normal Krebs solution, and transferred to fresh Krebs every 20 minutes for 100 minutes. All these solutions were discarded. At 100 minutes all tissues were placed in normal Krebs solution for 10 minutes, transferred to normal Krebs again for a second 10 minutes, placed in the appropriate drug solution for 10 minutes and back into normal Krebs for a final 10 minutes. A 0.5 ml aliquot of the final four solutions were counted as were all tissues.

F. Calculation and Expression of Results of Efflux Experiments

For each tissue the total $[^3H]$-noradrenaline content at time zero could be determined by back-addition of the dpm in the efflux medium at the end of each time interval to the counts remaining in the tissue at the end of the experiment. Then the rate of efflux of $[^3H]$-noradrenaline was determined, as were the counts remaining in the tissue at a given time (or tissue desaturation). $[^3H]$-noradrenaline efflux was then expressed as a rate coefficient combining these two values and given by equation (6):

$$(6) \quad E_{co} = \frac{\text{Rate of efflux } (\Delta \text{ dpm}/\Delta \text{ t})}{\text{Counts remaining at midpoint of time interval, t}}$$

The E_{co} for each tissue was then plotted against time. The E_{co} is a useful measure since it reflects alterations in either rate of efflux or the tissue desaturation.

After sample counts were corrected for efficiency and the appropriate aliquot factor, the data was processed as described above by means of an APL computer programme, designed for the analysis of efflux data (Cook and Taylor, 1971).

In the next chapter (Results), * marked between two points on a graph or between two bars on a histogram, indicates that a significant difference ($p < 0.05$, Student's paired or unpaired t-test) exists between the points or the bars.

Standard errors were not included in figures containing five dose-response curves, in the interest of clarity. These were included in all other figures, however.

RESULTS

I CONTRACTILITY STUDIES

A. Spontaneous Activity

Isolated spiral strips of rabbit portal vein almost invariably inhibited spontaneous contractile activity. When present, it was detectable soon after the tissues were set up, and was well-established when experiments were begun 60 minutes later. The spontaneous activity was found to vary widely in both frequency and amplitude, but once these characteristics were established for each tissue, they were maintained for the duration of the experiment. Recordings demonstrating some examples of spontaneous activity are shown in Fig. 13a. The frequency of spontaneous activity was found to vary from approximately 3 per minute to 10 per minute, while the amplitude varied from about 2% to 15% of the maximum contraction to noradrenaline (the most potent agonist in this preparation).

The spontaneous activity very seldom interferred with the measurement of the response of the tissues to drugs. In most cases, when supra-threshold concentrations of drug were added a tetanus-like contractile response was produced and the superimposed spontaneous activity was greatly reduced or abolished. At near-maximum and maximum concentrations of agonist, there was no trace of spontaneous activity associated with the contraction to the drug (Fig. 13b).

B. Responses to Agonists

Histamine produced dose-dependent contractions of isolated spiral strips of rabbit portal vein (Fig. 14). These occurred over a high dose range, (from 10^{-5}M to 10^{-2}M), and exhibited an unusual characteristic. When measured at hourly intervals for up to 5 hours in normal tissues, the histamine response was found to sensitize with time, reaching a

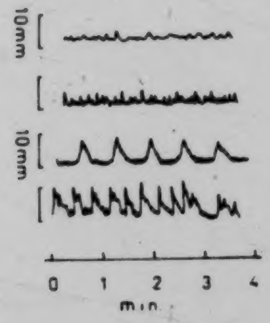


FIG. 13a Some examples of spontaneous activity of isolated spiral strips of rabbit portal vein.

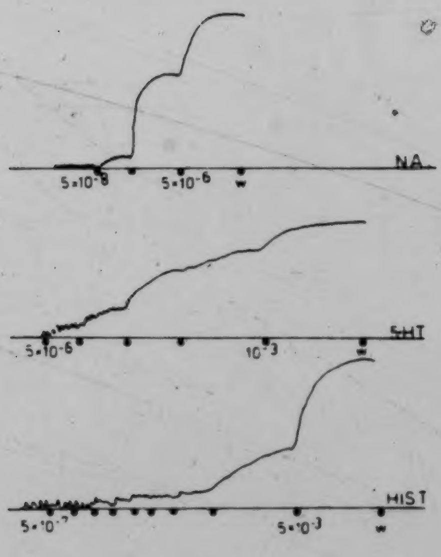


FIG. 13b Recordings showing typical cumulative dose-response curves to noradrenaline (NA), 5-HT and histamine (HIST), demonstrating the disappearance of spontaneous activity with increasing drug concentration. Dots indicate addition of drug, W refers to wash.

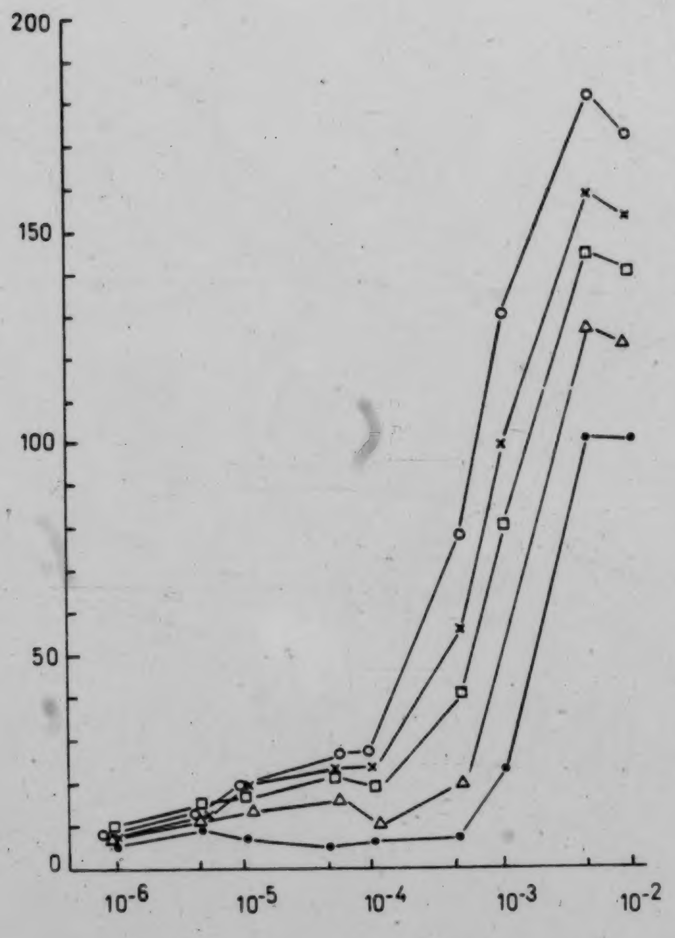


FIG. 14 Dose-response curves to histamine in untreated tissues, measured at one hour intervals for 5 hours. All results expressed as a percentage of initial maximum response at one hour. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 14.

● - ● : initial dose-response curve to histamine.
Δ - Δ : histamine response at 2 hours.
◻ - ◻ : histamine response at 3 hours.
x - x : histamine response at 4 hours.
o - o : histamine response at 5 hours.

maximum contraction at 5 hours almost 75% greater in amplitude than the initial maximum contraction at one hour.

Histamine was also found to produce dose-dependent contractions of tissues taken from rabbits which had been pre-treated with reserpine (Fig. 15). The contractions occurred over the same dose-range as those in normal tissues, but in this case, the response to histamine sensitized to a lesser extent. In reserpinized tissues, the maximum contraction to histamine at 5 hours was found to be only 25% greater in amplitude than the maximum contraction at one hour.

The responses of spiral strips of untreated portal vein to noradrenaline (Fig. 16) and acetylcholine (Fig. 17) differed from those to histamine. The noradrenaline dose-response curve occurred over a dose-range of 10^{-8}M to 10^{-5}M, and showed no sensitization with time, while the response to acetylcholine occurred over a dose-range of 10^{-7}M to 5 x 10^{-4}M, and showed only a small amount of sensitization. The maximum response to acetylcholine after 5 hours was found to be only 10% greater than the initial maximum response at one hour, and did not differ significantly from control.

The examination of the response of rabbit portal vein to histamine thus has two components, which will be dealt with separately. The first component, discussed in the following sections, concerns the characterization of the receptor for histamine. The second component, the examination of the mechanism of sensitization to histamine, will be considered later.

C. Blockade with Antagonists

In order to determine the nature of the receptor for histamine in rabbit portal vein, an attempt was made to block the response to this

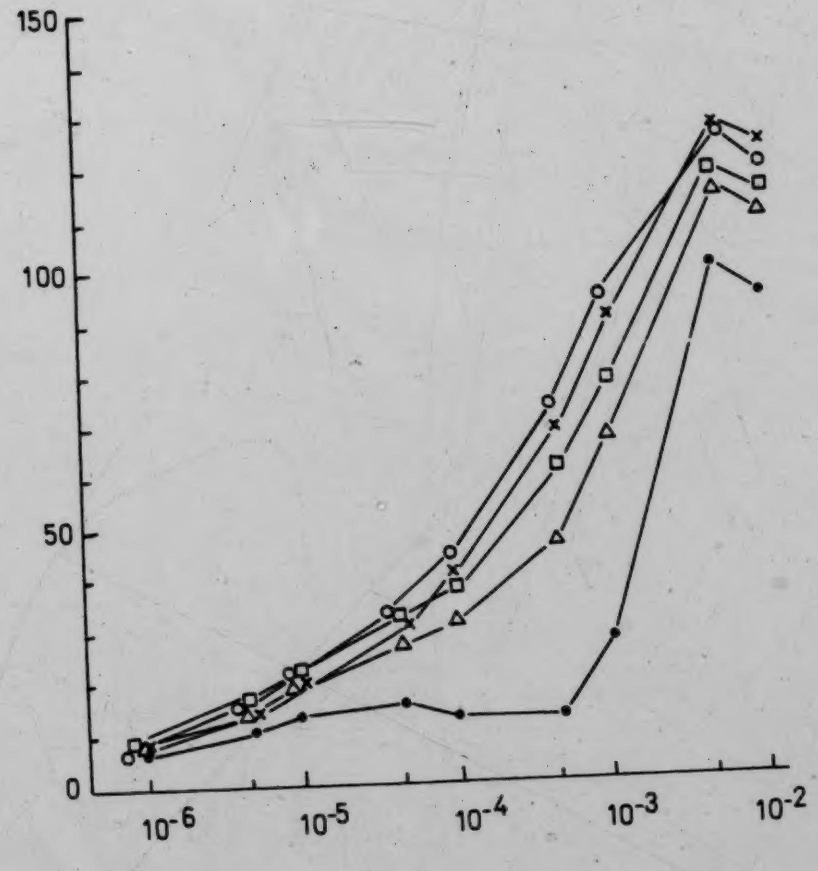


FIG. 15 Dose-response curves to histamine in reserpinized tissues, measured at one hour intervals for 5 hours. All results expressed as a percentage of initial maximum response at one hour. Log molar concentration of histamine as abscissa. per cent maximum response as ordinate. n = 13.

● – ● : initial dose-response curve to histamine.
Δ – Δ : histamine response at 2 hours.
□ – □ : histamine response at 3 hours.
x – x : histamine response at 4 hours.
o – o : histamine response at 5 hours.

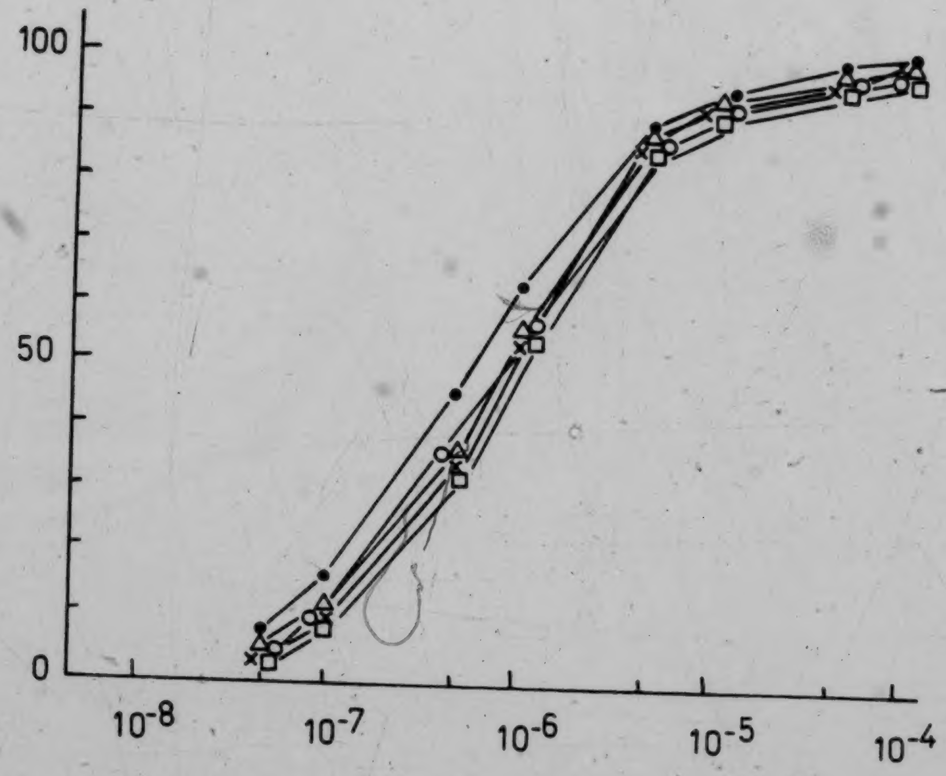


FIG. 16 Dose-response curves to noradrenaline in untreated tissues, measured at one hour intervals for 5 hours. All results expressed as a percentage of initial maximum response at one hour. Log molar concentration as abscissa, percent maximum response as ordinate. n = 8.

● – ● : initial dose-response curve to noradrenaline.
Δ – Δ : noradrenaline response at 2 hours.
□ – □ : noradrenaline response at 3 hours.
x – x : noradrenaline response at 4 hours.
o – o : noradrenaline response at 5 hours.

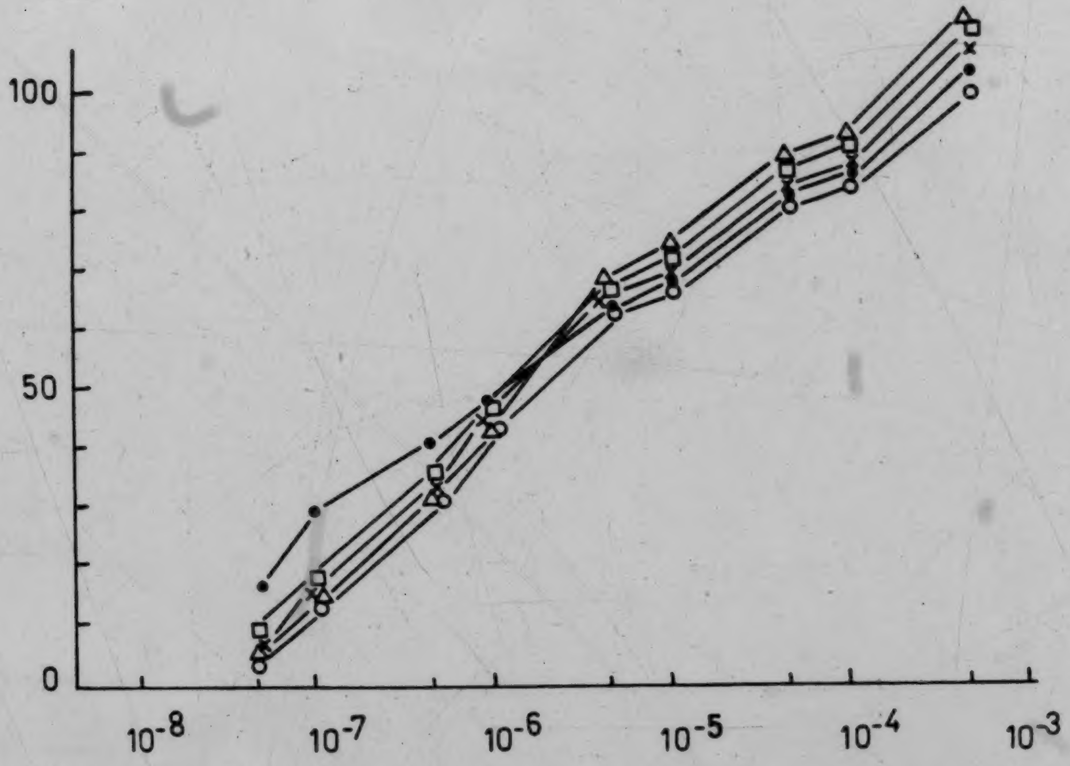


FIG. 17 Dose-response curves to acetylcholine in untreated tissues, measured at one hour intervals for 5 hours. All results expressed as a percentage of initial maximum response at one hour. Log molar concentration as abscissa, per cent maximum response as ordinate. n = 8.

● - ● : initial dose-response curve to acetylcholine.
Δ - Δ : acetylcholine response at 2 hours.
□ - □ : acetylcholine response at 3 hours.
x - x : acetylcholine response at 4 hours.
o - o : acetylcholine response at 5 hours.

agonist with the classical H_1 antagonists, diphenhydramine and chlorpheniramine. High concentrations of these antagonists were required to block the histamine response, and the blockade produced appeared to be non-competitive (Figs. 18,19). It is difficult to ascertain the nature of the antagonism produced by these agents, since the tissue does not respond in a reproducible manner to the extremely high concentrations of histamine required in the presence of antagonist.

In order to determine whether the blockade produced by these agents was specific for the histamine response, these same concentrations of antagonist were used in an attempt to block the noradrenaline response. Chlorpheniramine had no effect on the response to noradrenaline (Fig. 20), and neither did the lower concentration of diphenhydramine (10^{-6}M), although 5 x 10^{-6}M diphenhydramine appeared to produce a small alteration of the response to noradrenaline (Fig. 21).

The histamine H_2 receptor in guinea pig heart has been reported to be blocked in an apparently non-competitive manner by high concentrations of H_1 antagonists (McNeill and Verma, 1974b). In view of these results, attempts were made to block the histamine response in rabbit portal vein with the H_2 antagonist metiamide (Fig. 22). This antagonist was effective only in high concentrations, producing a similar type of antagonism to that seen with the H_1 antagonists, and as will become apparent, seen with all other antagonists of the histamine response. The blockade produced by metiamide however, was not specific for histamine, since the acetylcholine response was significantly depressed by the same doses of this antagonist (Fig. 23).

Further attempts to block the histamine receptor in portal vein led to the discovery that the α-adrenergic antagonist phentolamine was

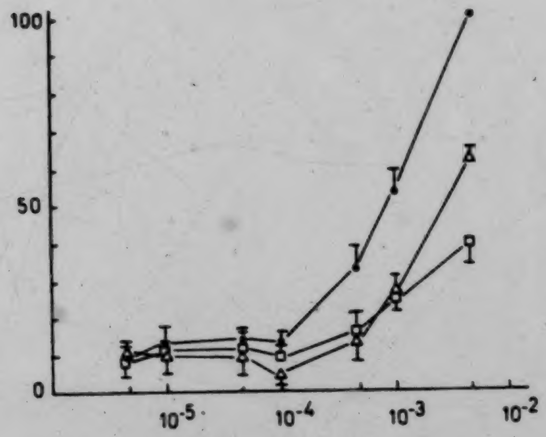


FIG. 18 The effect of diphenhydramine on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : control dose-response curve to histamine.
Δ Δ : histamine response after 10^{-6}M diphenhydramine.
□ - □ : histamine response after 5 x 10^{-6}M diphenhydramine.

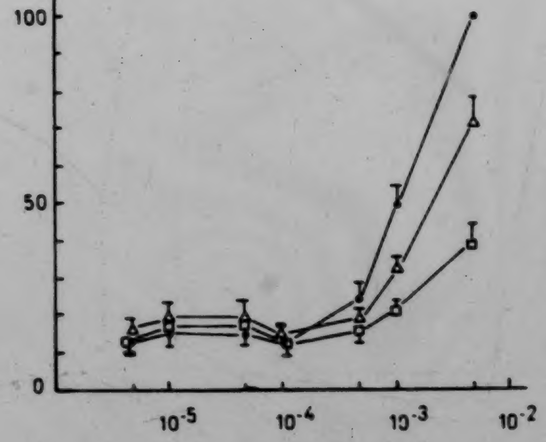


FIG. 19 The effect of chlorpheniramine on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : control dose-response curve to histamine.
Δ - Δ : histamine response after 10^{-6}M chlorpheniramine.
□ - □ : histamine response after 5 x 10^{-6}M chlorpheniramine.

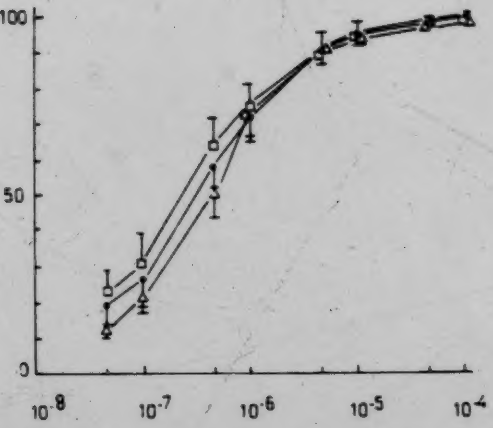


FIG. 20 The effect of chlorpheniramine on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-6}M chlorpheniramine.
□ - □ : noradrenaline response after 5 x 10^{-6}M chlorpheniramine.

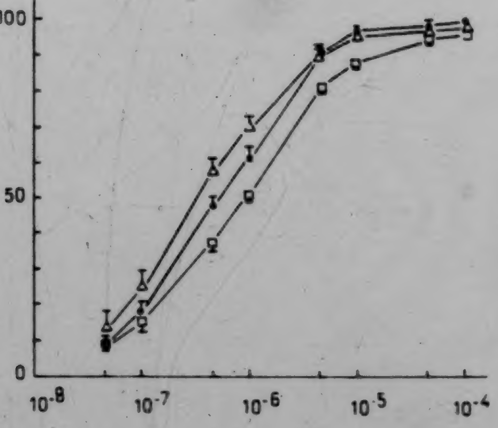


FIG. 21 The effect of diphenhydramine on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 4. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-6}M diphenhydramine.
□ - □ : noradrenaline response after 5 x 10^{-6}M diphenhydramine.

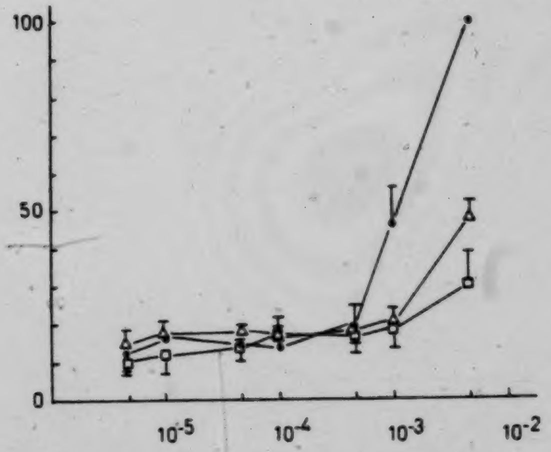


FIG. 22 The effect of metiamide on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● – ● : control dose-response curve to histamine.
Δ – Δ : histamine response after 10^{-4}M metiamide.
□ – □ : histamine response after 5 x 10^{-4}M metiamide.

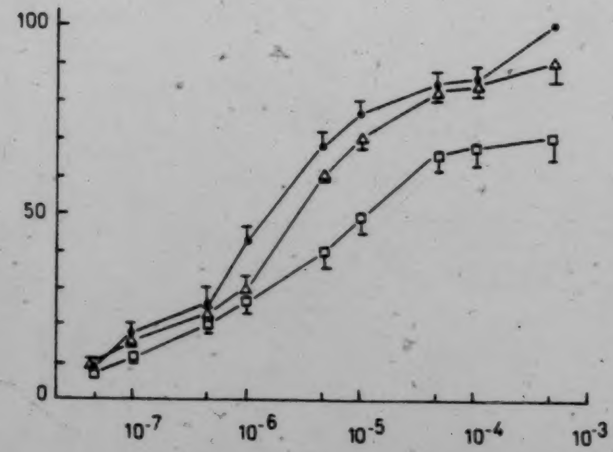


FIG. 23 The effect of metiamide on the response to acetylcholine. Log molar concentration of acetylcholine as abscissa, per cent maximum response as ordinate. n = 4. Bars represent standard errors.

● – ● : control dose-response curve to acetylcholine.
Δ – Δ : acetylcholine response after 10^{-4}M metiamide.
□ – □ : acetylcholine response after 5 x 10^{-4}M metiamide.

also an effective antagonist of the histamine response. A concentration of phentolamine (10^{-8}M), which had almost no effect on the response to noradrenaline (Fig. 24), caused a reduction of close to 60% in the maximum response to histamine (Fig. 25). Similarly, a concentration of phentolamine (10^{-7}M) which caused less than a one log unit shift in the noradrenaline dose-response curve, reduced the maximum response to histamine to about 40% of the control value.

The histamine response was unaffected by atropine (Fig. 26), in a concentration (10^{-7}M) sufficient to alter the acetylcholine response to a significant extent (Fig. 27). Fig. 27 illustrates an interesting feature of the response to acetylcholine: the maximum response, at 5×10^{-4}M is insensitive to blockade by atropine, even in concentrations as high as 5×10^{-7}M. The response to this concentration of acetylcholine is thought to result from release of noradrenaline, since it is blocked by phentolamine and reduced or abolished in reserpinized tissues. Release of noradrenaline by high concentrations of acetylcholine has been reported (Burn, 1977). The mechanism of the response to high doses of acetylcholine was not examined further in this study. Acetylcholine was occasionally used as a control in the experiments to be described in the following sections, however, and to ensure that this agent was acting through the cholinergic receptor, a concentration of acetylcholine (5×10^{-5}M) was employed which was very sensitive to atropine.

D. Mechanism of Action of Histamine

The initial dose-response curve to histamine in reserpinized tissues was not significantly different from the control, although reserpinized tissues sensitized less than untreated tissues. This led

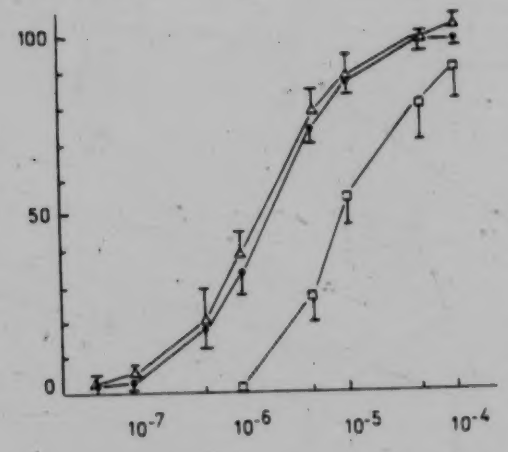


FIG. 24 The effect of phentolamine on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 4. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-8}M phentolamine.
□ - □ : noradrenaline response after 10^{-7}M phentolamine.

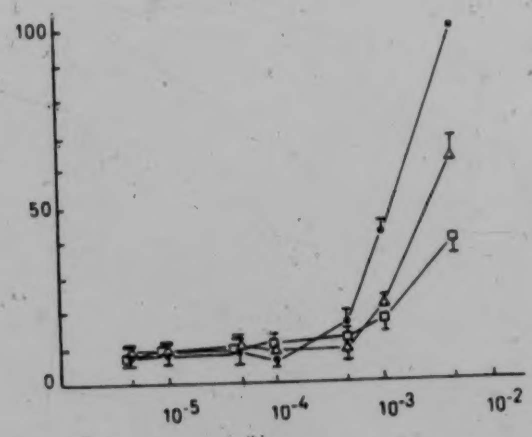


FIG. 25 The effect of phentolamine on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : histamine response after 10^{-8}M phentolamine.
□ - □ : histamine response after 10^{-7}M phentolamine.

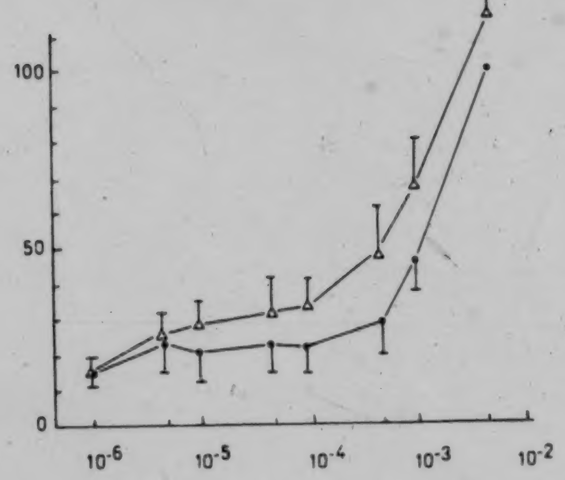


FIG. 26 The effect of atropine on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : initial dose-response curve to histamine.
Δ - Δ : histamine response after 10^{-7}M atropine.

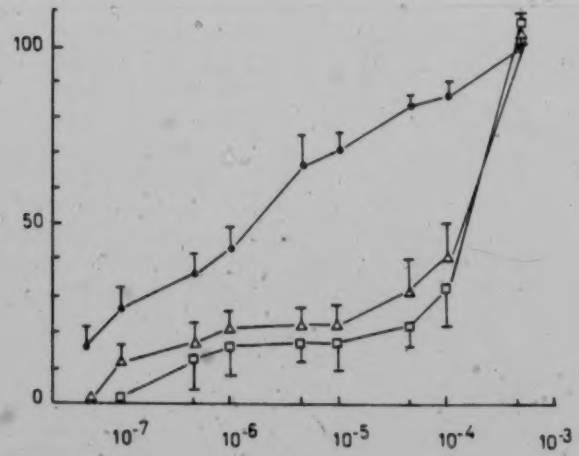


FIG. 27 The effect of atropine on the response to acetylcholine. Log molar concentration of acetylcholine as abscissa, per cent maximum response as ordinate. n = 5. Bars represent standard errors.

● - ● : control dose-response curve to acetylcholine.
Δ - Δ : acetylcholine response after 10^{-7}M atropine.
□ - □ : acetylcholine response after 5 x 10^{-7}M atropine.

to the conclusion that histamine was acting directly on smooth muscle, rather than through the release of noradrenaline, in this preparation. However, reserpinized tissues were then found to respond to tyramine (Fig. 28), a sympathomimetic amine known to act mainly through release of noradrenaline (Trendelenburg _et_ _al_., 1962). The response to tyramine persisted in portal veins taken from rabbits pre-treated with twice the suggested concentration of reserpine (4.0 mg/kg 16 hours, and 2.0 mg/kg one hour before being killed. The normal dose is 2.0 mg/kg 16 hours and 1.0 mg/kg one hour before sacrifice).

The possibility that histamine was acting indirectly through release of noradrenaline could thus not be discounted on the basis of studies in reserpinized tissues, and a number of approaches were taken to examine this possibility further. The first was to induce tachyphylaxis to a high concentration of tyramine, and examine the effect of this procedure on the response to histamine. The results are shown in Fig. 29; even when the response to tyramine (10^{-3}M) is reduced to less than 20% of the control value, the response to histamine (5×10^{-3}M) remains unaffected.

Guanethidine (2×10^{-5}M) was also used, since it is known to be an adrenergic neuron blocking agent which inhibits responses to sympathetic stimulation (Boura and Green, 1965). This agent reduced the response to electrical stimulation to about 35% of the control value, but had only a small effect on the response to 10^{-3}M tyramine, reducing it to 85% of the control value. The response to histamine was unaffected by this treatment (Fig. 30). Since the tyramine response was only minimally reduced by guanethidine, it was thought that tyramine might be having a direct action on smooth muscle at this concentration (10^{-3}M),

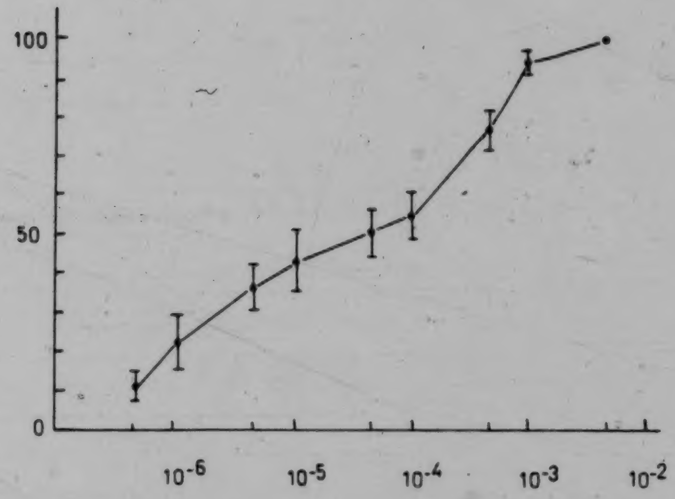


FIG. 28 Dose-response curve to tyramine obtained on reserpinized tissues. Log molar concentration of tyramine as abscissa, per cent maximum response as ordinate. n = 4. Bars represent standard errors.

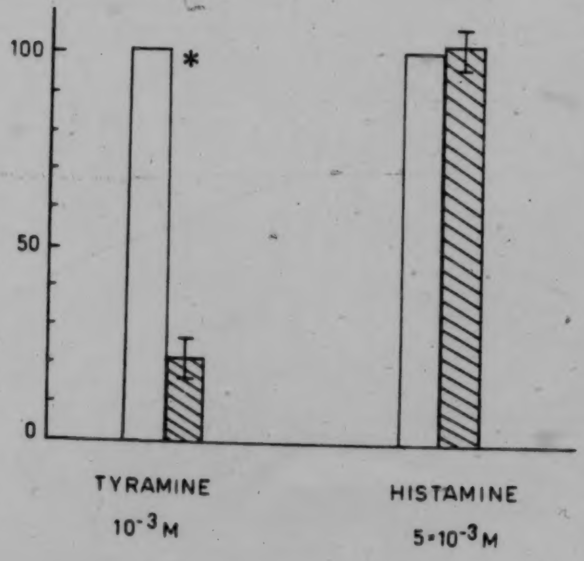


FIG. 29 Histogram illustrating the effects of induction of tachyphylaxis to tyramine on the responses to histamine and tyramine. Per cent maximum response as ordinate. n = 4. Bars represent standard errors.

☐ : control response.

▨ : response after induction of tachyphylaxis to tyramine.

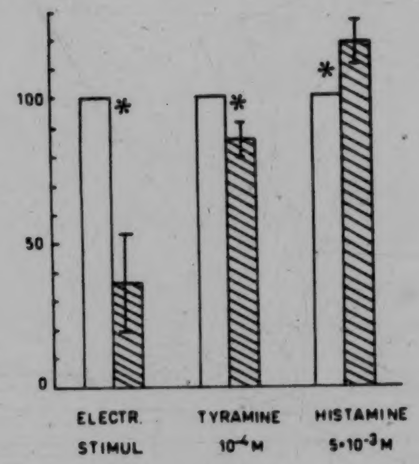


FIG. 30 Histogram illustrating the effect of guanethidine on the responses to electrical stimulation, (ELECTR. STIMUL), tyramine and histamine. Per cent maximum response as ordinate. n = 9. (Electrical stimulation, n = 3). Bars represent standard errors.

□ : control response.

▨ : response after 3 x 10^{-5}M guanethidine.

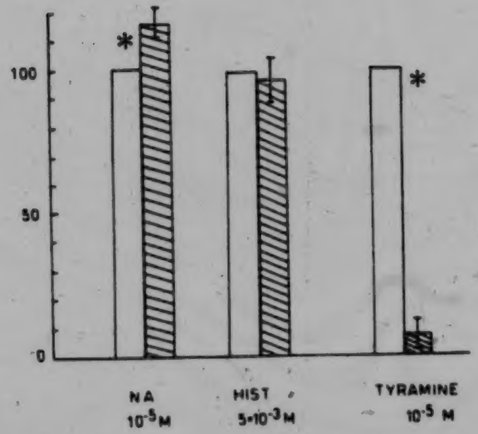


FIG. 31 Histogram illustrating the effect of cocaine on the responses to noradrenaline (NA), histamine (HIST) and tyramine. Per cent maximum response as ordinate. n = 9. Bars represent standard errors.

□ : control response.

▨ : response after 3 x 10^{-5}M cocaine.

and it was decided to decrease the tyramine concentration to 10^{-4}M or 10^{-5}M in the remaining experiments. The possibility also existed however, that guanethidine might not be causing sufficient depletion of the noradrenaline stores. In addition to its reserpine-like action, guanethidine appears to function as a local anesthetic, selective for adrenergic nerve terminals (Haeusler et al., 1969). It could thus antagonize the effects of nerve stimulation while producing only a small inhibition of the response to sympathomimetic agents. It was therefore decided that other procedures would be necessary to demonstrate clearly the presence or absence of an indirect action of histamine at adrenergic nerve terminals.

In the presence of 3 x 10^{-5}M cocaine, which prevents the neuronal uptake of noradrenaline and tyramine (Iverson, 1967), the response to 10^{-5}M tyramine was reduced to about 10% of the control response (Fig. 31). This treatment had no effect on the response to histamine, while the response to noradrenaline was significantly increased. The response to 10^{-4}M tyramine (not shown) was reduced to about 40% of control.

Treatment of portal veins with 6-hydroxydopamine (6-OHDA), an agent which causes selective degeneration of adrenergic nerves (Malmfors and Sachs, 1968), abolished the response to 10^{-4}M tyramine (Fig. 32). This treatment also reduced the response to histamine, but the response to acetylcholine (5 x 10^{-5}M) was reduced by a similar amount, suggesting that 6-OHDA could be exerting a non-specific effect on the smooth muscle of the tissue.

The experiments discussed above strongly imply that histamine is not acting through release of noradrenaline. Since the response to

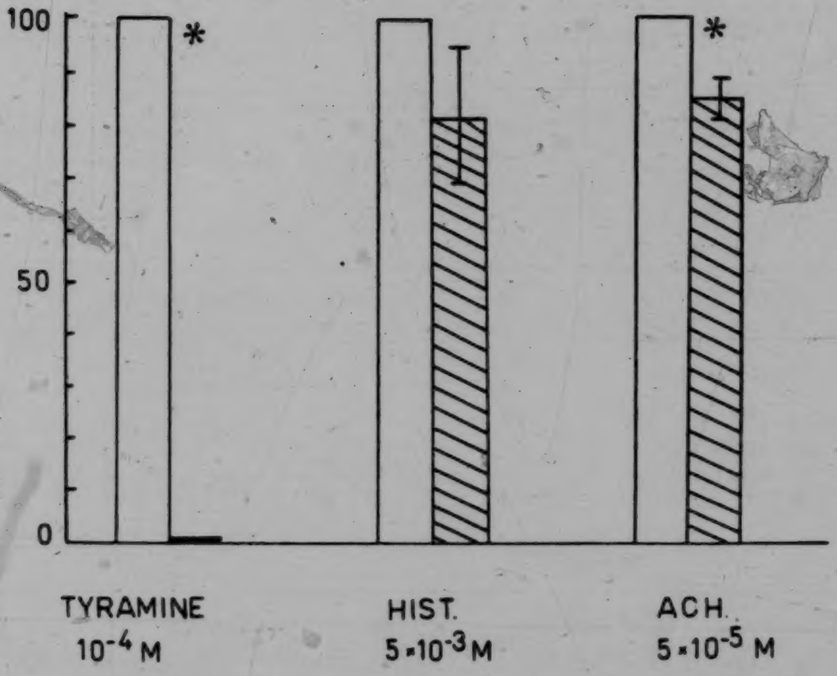


FIG. 32 Histogram illustrating the effects of 6-OHDA on the responses to tyramine, histamine (HIST) and acetylcholine (ACH). Per cent maximum as ordinate. n = 4. Bars represent standard errors.

☐ : control response.

▨ : response after 250 μg/ml 6-OHDA.

histamine is also insensitive to atropine, it is reasonable to suggest that responses to this agonist are mediated by a direct action on the smooth muscle of the portal vein.

E. Receptor Differentiation Through Antagonism

Since experiments discussed in the previous section had suggested a direct action of histamine at a receptor in rabbit portal vein, attempts were made to determine the nature of this receptor. The antagonism of the histamine response by phentolamine suggested that histamine was acting either at a novel type of histamine receptor, or at a receptor at which noradrenaline was also acting. In order to distinguish between these possibilities, attempts were made to block the responses to these two agonists with a variety of different antagonists. Normally the pA_2 values of the various antagonists against histamine and noradrenaline would be used to determine if these agonists were active at the same receptor (Arunlakshana and Schild, 1959). This method could not be used in this case however, since it requires that the antagonism produced be competitive in nature, and as mentioned earlier, the type of antagonism produced against the histamine response cannot be determined. Thus the only means of distinguishing between the receptors for these two agonists would be on the basis of selective antagonism, if an antagonist blocked the response to one agonist but not the other.

The first antagonist chosen was antazoline, an H_1-antagonist which is structurally very similar to phentolamine (Figs. 33,34). Antazoline was found to block the histamine response in concentrations (10^{-6}M and 5×10^{-6}M) similar to those required by the other H_1-antagonists employed (Fig. 35). In contrast to the earlier results however, the

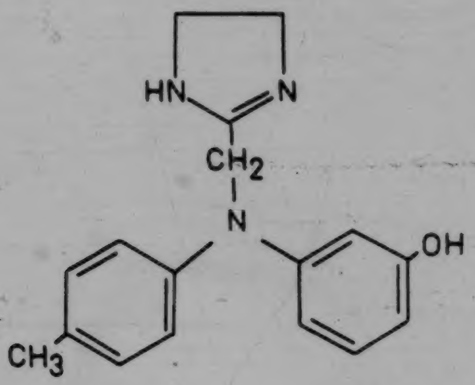

HN
N
CH_2
N
OH
CH_3

FIG. 33 The chemical structure of phentolamine.

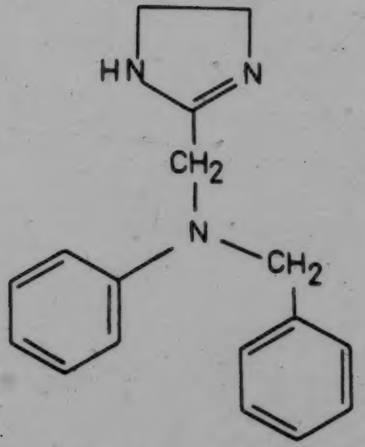

HN
N
CH_2
N
CH_2

FIG. 34 The chemical structure of antazoline.

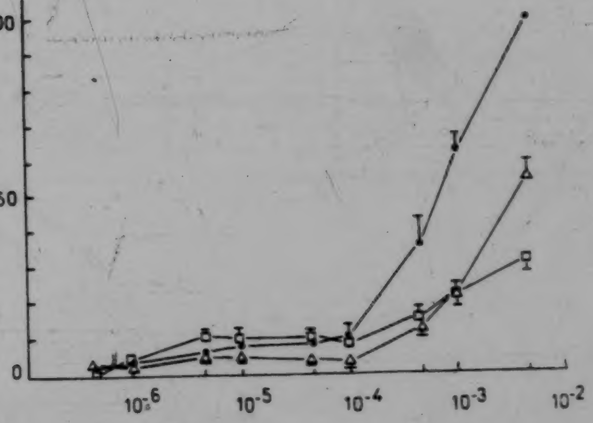


FIG. 35 The effect of antazoline on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 9. Bars represent standard errors.

● - ● : control dose-response curve to histamine.
Δ - Δ : histamine response after 10^{-6}M antazoline.
□ - □ : histamine response after 5 x 10^{-6}M antazoline.

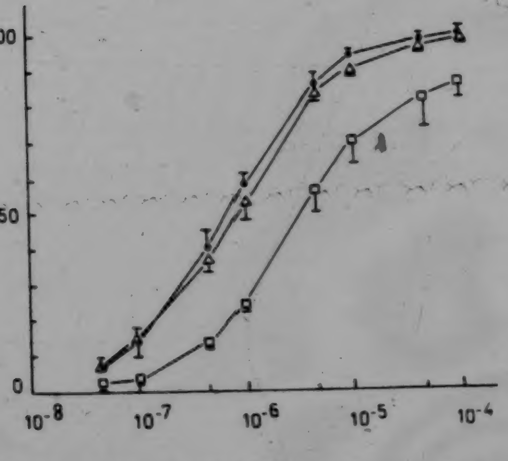


FIG. 36 The effect of antazoline on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-6}M antazoline.
□ - □ : noradrenaline response after 5 x 10^{-6}M antazoline.

noradrenaline response was also significantly altered by antazoline (Fig. 36).

Efforts were next made to block the responses to histamine and noradrenaline with a number of α-adrenergic antagonists. Those used were tolazoline, azapetine and dibozane.

Tolazoline, another imidazoline structurally similar to phentolamine (Fig. 37), acted as an agonist in this tissue (see next section). Azapetine (Fig. 38) and dibozane (Fig. 39) were effective antagonists of the response to noradrenaline in this preparation, (Figs. 40,41), but in the same concentrations (10^{-8}M, 10^{-7}M) that were employed to block this response, both these antagonists were also effective at blocking the histamine response (Figs. 42,43).

It was thus impossible to distinguish between the receptor for histamine and the receptor for noradrenaline on the basis of studies with reversible antagonists. It was felt however, that examination of the action of drugs which act as agonists at histamine receptors in other preparations might provide some information about the nature of the histamine receptor in portal vein.

F. Responses to Other Agonists

Tolazoline was originally classified as an α-adrenergic antagonist, although even then its histamine-like actions were recognized (Nickerson, 1949). Recently, tolazoline has been reported to act as an agonist, both at H_2 receptors and at α-adrenergic receptors (Saunders et al., 1975; Yellin et al., 1975).

Betazole is an isomer of histamine, that has been reported to be more active at H_2 than at H_1 receptors (Rosiere and Grossman, 1952).

Both these agents cause dose-dependent contractions of rabbit

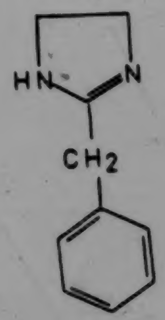


FIG. 37 The chemical structure of tolazoline.

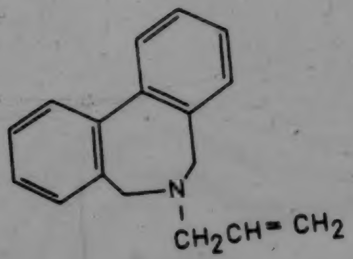


FIG. 38 The chemical structure of azapetine.

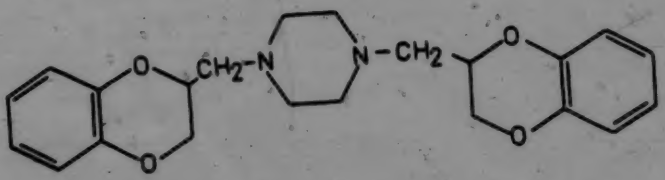

O
O
CH2-N
N-CH2
O
O

FIG. 39 The chemical structure of dibozane.

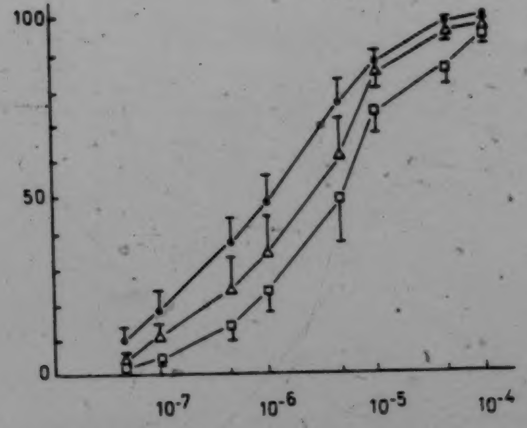


FIG. 40 The effect of azapetine on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 7. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-8}M azapetine.
▫ - ▫ : noradrenaline response after 10^{-7}M azapetine.

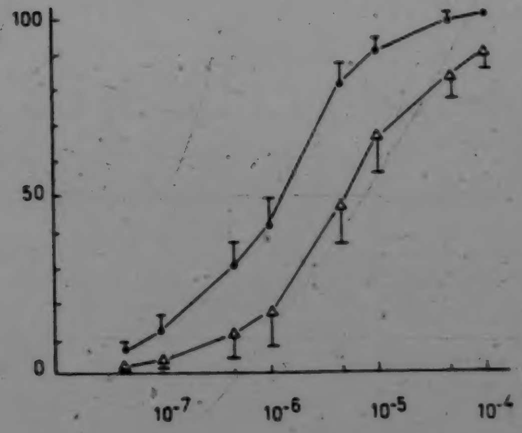


FIG. 41 The effect of dibozane on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 11. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-8}M dibozane.

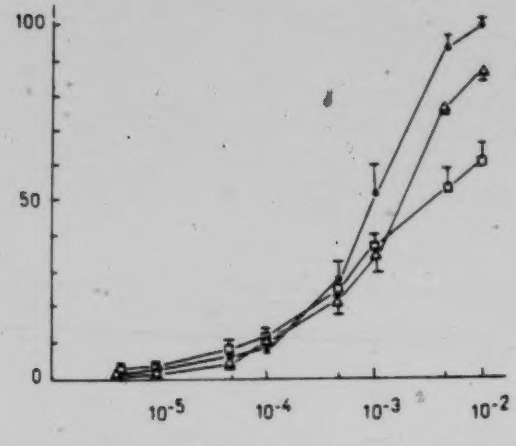


FIG. 42 The effect of azapetine on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● – ● : control dose-response curve to histamine.
Δ – Δ : histamine response after 10^{-8}M azapetine.
□ – □ : histamine response after 10^{-7}M azapetine.

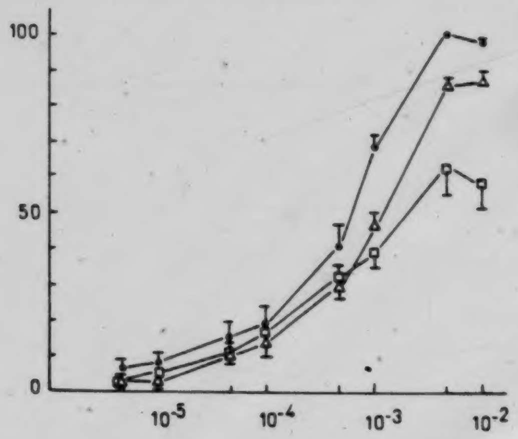


FIG. 43 The effect of dibozane on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 7. Bars represent standard errors.

● – ● : control dose-response curve to histamine.
Δ – Δ : histamine response after 10^{-8}M dibozane.
□ – □ : histamine response after 10^{-7}M dibozane.

vein, tolazoline over a dose-range of 10^{-7}M to 10^{-4}M (Fig. 44), and betazole over a dose-range of 10^{-6}M to 5 x 10^{-3}M (Fig. 45). The responses to both these agonists are quite variable, but they show some sensitization with time in untreated tissues. Treatment of tissues with 6-OHDA had no effect on the responses to these agents (Fig. 46), nor was the tolazoline response affected by cocaine. Studies with antagonists indicated that as was the case with histamine, phentolamine was the most effective antagonist of the responses to these two agonists (Figs. 47,48).

These results indicate that betazole and tolazoline are acting on smooth muscle in this tissue, and may be acting at the same receptor as histamine, since responses to all three agonists can be blocked with phentolamine.

A comparison was next made of the effects of some histamine analogues, N-methyl-histamine (Fig. 49) and 4-methyl-histamine (Fig. 50), as well as ethyl-2-pyretamine (Fig. 51) with histamine on portal vein. N-methyl-histamine is reported to be more active at H_1 than H_2 receptors, while 4-methyl-histamine is more active at H_2 than H_1 receptors (Black et al., 1972). Ethyl-2-pyretamine is known to be a partial agonist at the H_1 receptor (Kenakin, 1975). In Fig. 52 the dose-response curves to these agonists are plotted as a percentage of the maximum response to histamine. The most effective agonist is N-methyl-histamine, while both 4-methyl-histamine and ethyl-2-pyretamine are almost inactive. Although further studies with antagonists were prevented by lack of sufficient quantities of these agonists, it seems apparent that no relation can be drawn between activity at either H_1 or H_2 receptors in other tissues, and activity in portal vein, whether or not it is at the

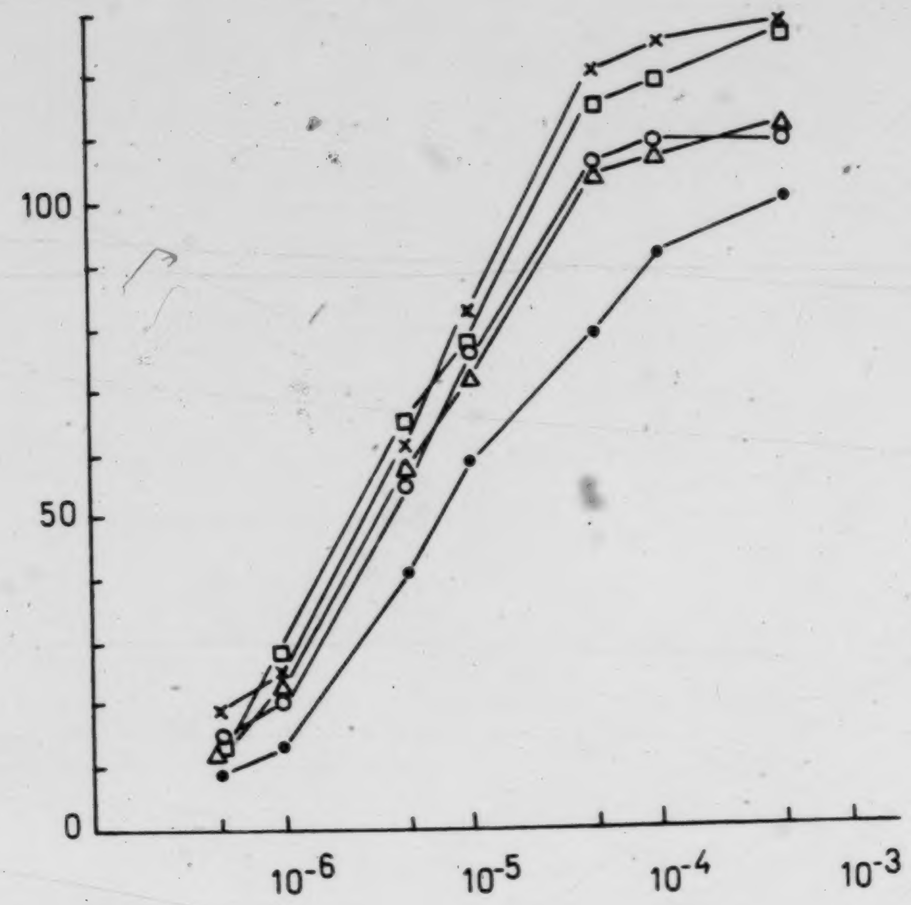


FIG. 44 Dose-response curves to tolazoline in untreated tissues, measured at one hour intervals for 5 hours. All results expressed as percentage of the initial maximum response at one hour. n = 10.

● - ● : initial dose-response curve to tolazoline
Δ - Δ : tolazoline response at 2 hours.
□ - □ : tolazoline response at 3 hours.
x - x : tolazoline response at 4 hours.
o - o : tolazoline response at 5 hours.

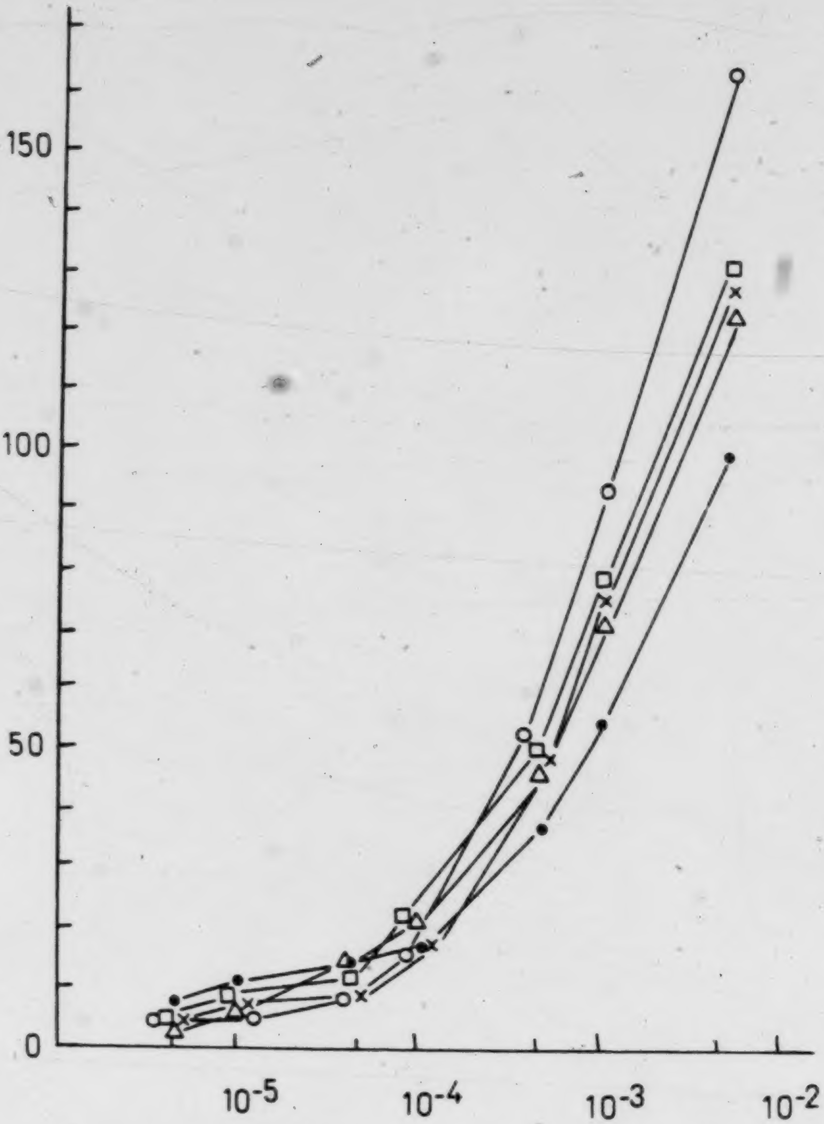


FIG. 45 Dose-response curves to betazole in untreated tissues, measured at one hour intervals for 5 hours. All results expressed as percentage of the initial maximum response at one hour. n = 9.

● - ● : initial dose-response curve to betazole.
Δ - Δ : betazole response at 2 hours.
□ - □ : betazole response at 3 hours.
x - x : betazole response at 4 hours.
o - o : betazole response at 5 hours.

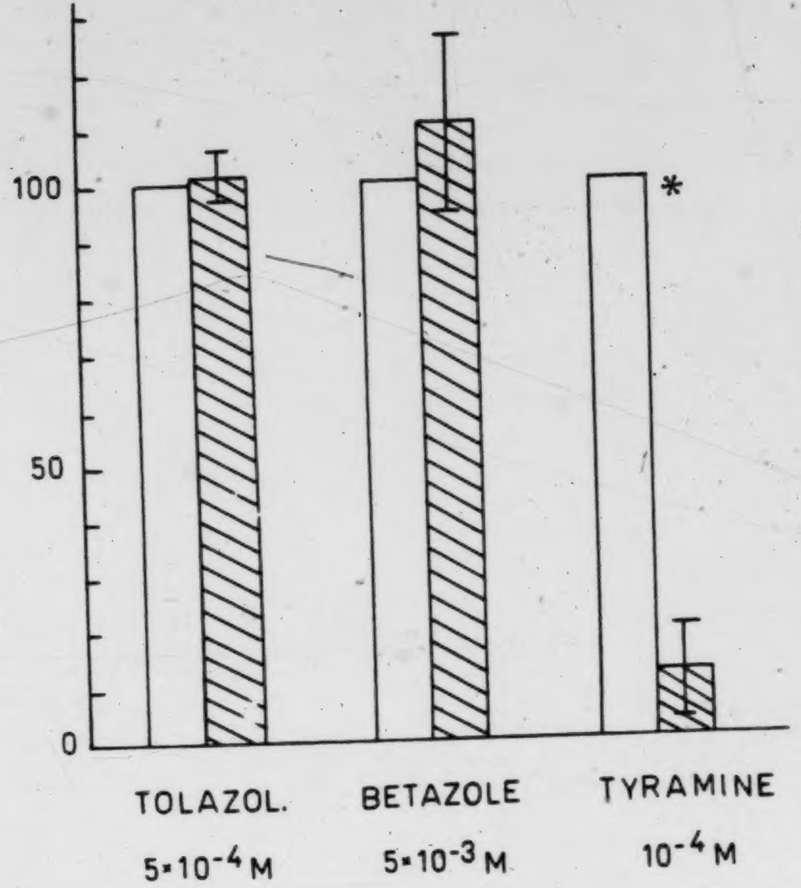


FIG. 46 Histogram illustrating the effect of 6-OHDA on the responses to betazole, tolazoline (tolazol.) and tyramine. Per cent maximum response as ordinate. n = 4. Bars represent standard errors.

□ : control response.

▨ : response after exposure to 250 μg/ml 6-OHDA.

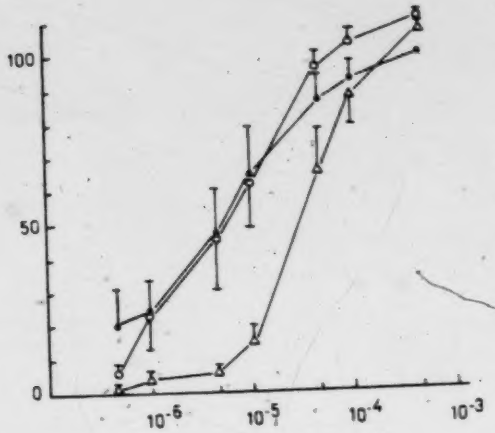


FIG. 47 The effect of phentolamine on the response to tolazoline. Log molar concentration of tolazoline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : control dose-response curve to tolazoline.
Δ - Δ : tolazoline response after 10^{-7}M phentolamine.
o - o : tolazoline response after a one hour wash with normal Krebs.

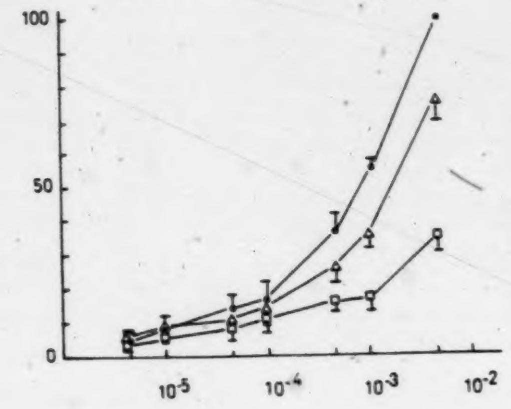


FIG. 48 The effect of phentolamine on the response to betazole. Log molar concentration of betazole as abscissa, per cent maximum response as ordinate. n = 10. Bars represent standard errors.

● - ● : control dose-response curve to betazole.
Δ - Δ : betazole response after 10^{-8}M phentolamine.
□ - □ : betazole response after 10^{-7}M phentolamine.

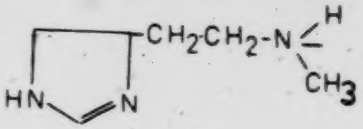


FIG. 49 The chemical structure of N-methyl-histamine.

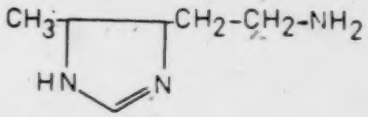


FIG. 50 The chemical structure of 4-methyl-histamine.

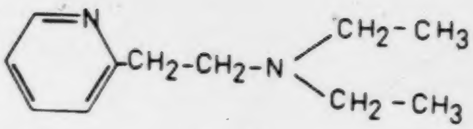


FIG. 51 The chemical structure of ethyl-2-pyretamine.

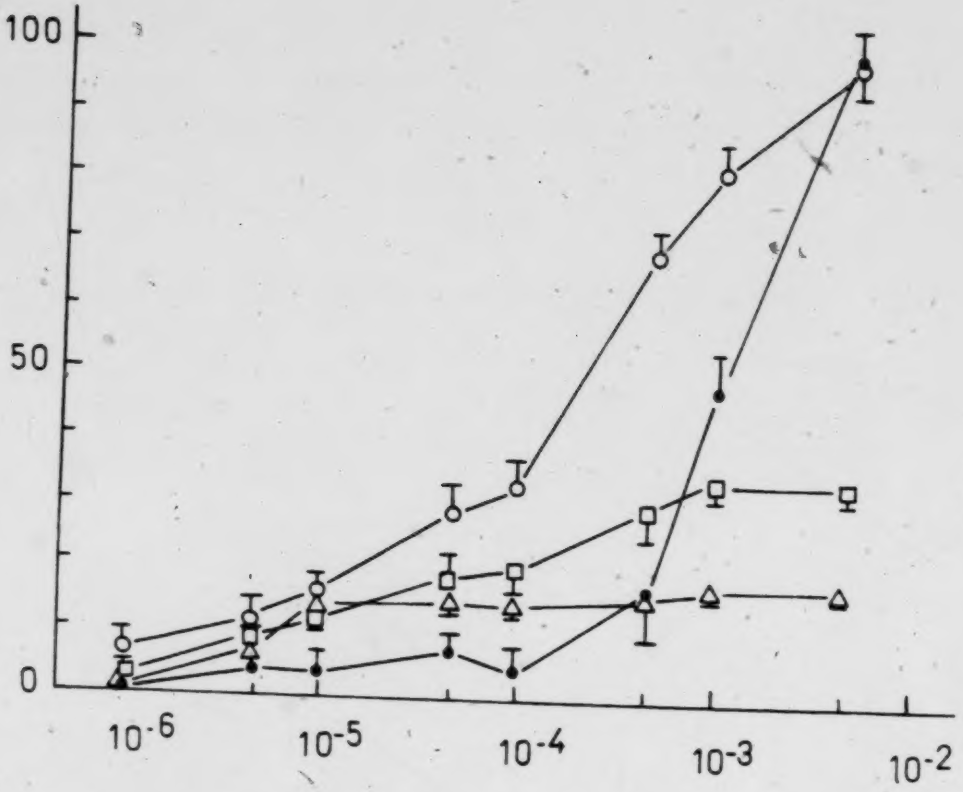


FIG. 52 Dose-response curves to histamine receptor agonists, obtained on the same preparation. All results expressed as a percentage of the maximum response to histamine. Log molar concentration as abscissa, per cent maximum response as ordinate. n = 4. Bars represent standard errors.

● - ● : dose-response curve to histamine.
o - o : dose-response curve to N-methyl-histamine.
□ - □ : dose-response curve to ethyl-2-pyretamine.
Δ - Δ : dose-response curve to 4-methyl-histamine.

same receptor as histamine.

G. Studies with 5-hydroxytryptamine

5-HT, like histamine, is normally assumed to act through specific receptors. These are classified as 'M' receptors, which are present in neuronal tissue and blocked by morphine, and 'D' receptors, present in smooth muscle and blocked by methysergide (Gaddum and Picarelli, 1957). 5-HT has also been shown to release noradrenaline in rabbit heart (Fozard and Mwaluko, 1976), and in other tissues (Innes, 1962b; Pluchino, 1972). Recently it has been reported that in addition to these actions, 5-HT can interact with the α-adrenergic receptor in rabbit ear artery, and this interaction could be blocked with phentolamine (Apperley et al., 1976). In view of these results, it was decided to examine the actions of 5-HT in portal vein, in order to determine whether 5-HT and histamine could be acting at the same receptor.

5-Hydroxytryptamine causes dose-dependent contractions of portal vein which occur over a dose-range of 5×10^{-7}M to 5×10^{-4}M (Fig. 53). Like the histamine response, the response to 5-HT shows sensitization with time, reaching a maximal response at 5 hours 60% greater than the initial maximum response at one hour.

Only the responses to low concentrations of 5-hydroxytryptamine are blocked with methysergide (10^{-6}M), although the sensitization seen in normal tissues is reduced in the presence of this antagonist (Fig. 54). Methylsergide also appears to reduce the sensitization seen to histamine, although it does not antagonize the response to this agonist (Fig. 55).

The 5-hydroxytryptamine response, to concentrations above 10^{-5}M, is blocked by phentolamine (10^{-7}M), which produces what appears to be a competitive type of antagonism (Fig. 56). In order to determine whether

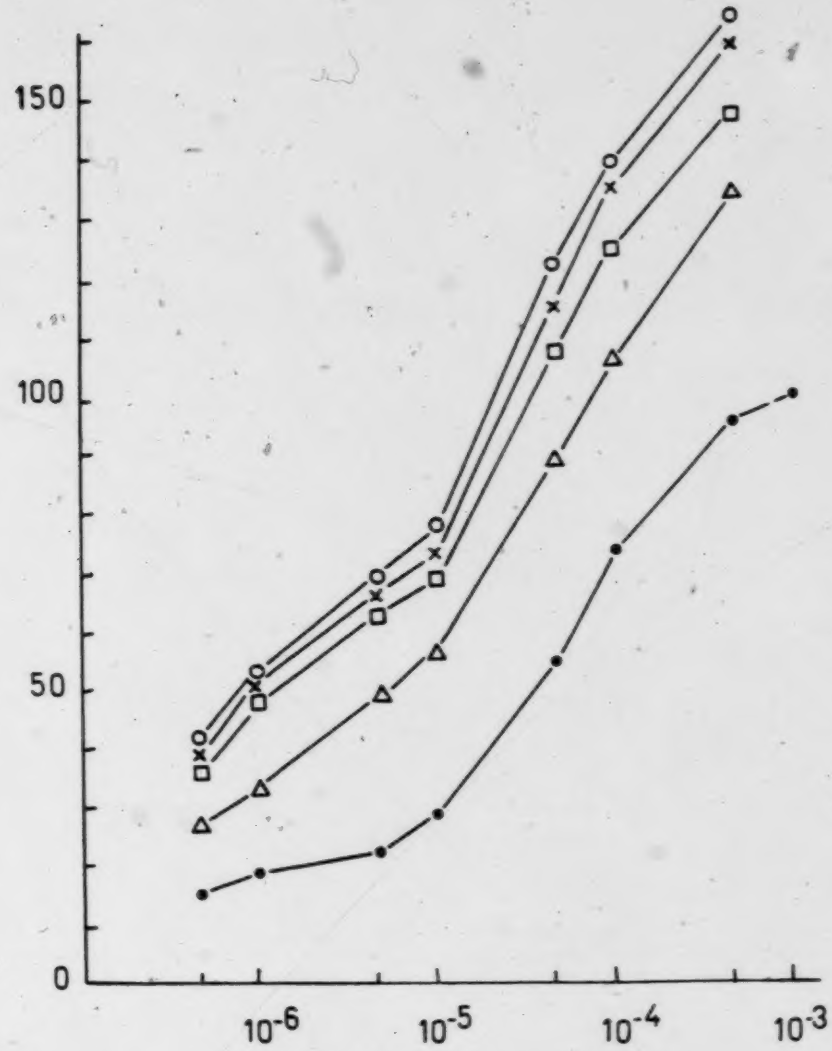


FIG. 53 Dose-response curves to 5-HT, measured at one hour intervals for 5 hours. All results expressed as a percentage of the initial maximum response at one hour. Log molar concentration as abscissa, per cent maximum response as ordinate. n = 8.

● - ● : initial dose-response curve to 5-HT.
Δ - Δ : 5-HT response at 2 hours.
□ - □ : 5-HT response at 3 hours.
x - x : 5-HT response at 4 hours.
o - o : 5-HT response at 5 hours.

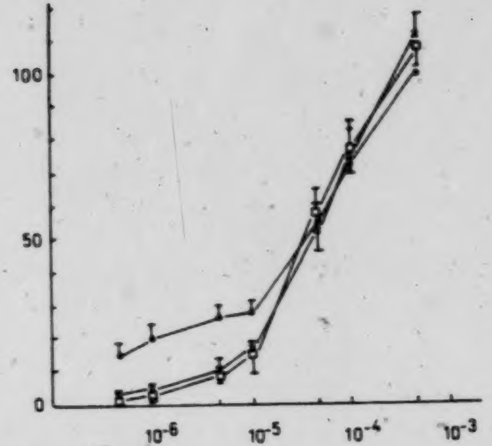


FIG. 54 The effect of methysergide on the response to 5-HT. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 9. Bars represent standard errors.

● - ● : control dose-response curve to 5-HT.
□ - □ : 5-HT response after 10^{-6}M methysergide for 2 hours.
x - x : 5-HT response after 10^{-6}M methysergide for 3 hours.

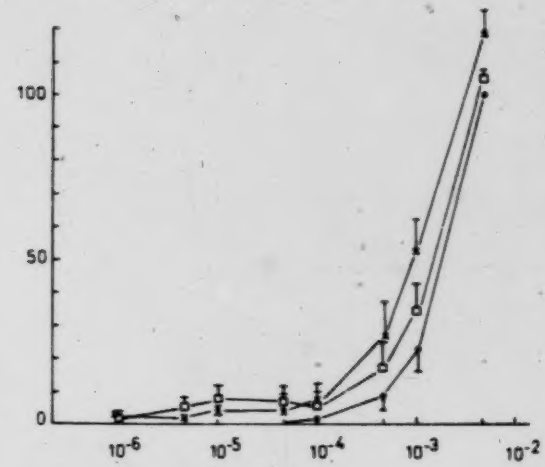


FIG. 55 The effect of methysergide on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : control dose-response curve to histamine.
□ - □ : histamine response after 10^{-6}M methysergide for 2 hours.
x - x : histamine response after 10^{-6}M methysergide for 3 hours.

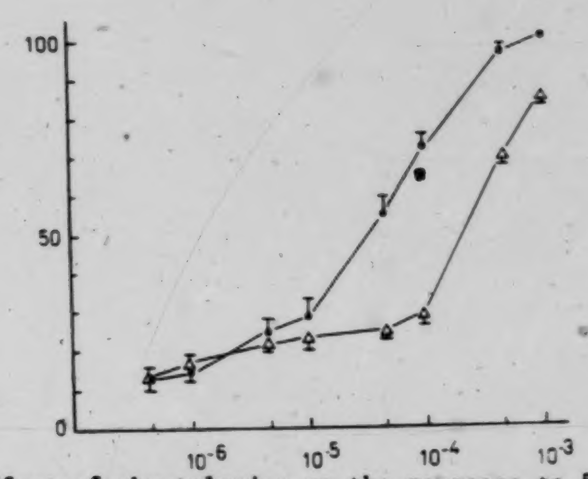


FIG. 56 The effect of phentolamine on the response to 5-HT. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : control dose-response curve to 5-HT.
Δ - Δ : 5-HT response after 10^{-7}M phentolamine.

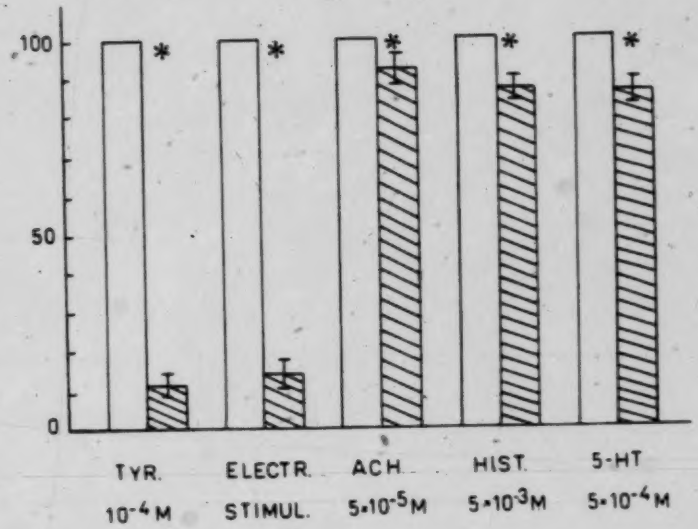


FIG. 57 Histogram illustrating the effect of 6-OHDA on the responses to tyramine (TYR), electrical stimulation (ELECTR. STIMUL.), acetylcholine (ACH), histamine (HIST) and 5-HT. Per cent maximum response as ordinate. n = 20, (electrical stimulation, n = 4). Bars represent standard errors.

☐ : control response
▨ : response after 250 μg/ml 6-OHDA.

5-HT is acting through release of noradrenaline, responses to this agonist were measured after treatment of the tissues with 6-OHDA (Fig. 57). This treatment, while reducing the responses to tyramine and to electrical stimulation to about 10% of the control value, had no selective effect on the response to 5-HT, the histamine and acetylcholine responses being reduced by the same amount.

These results imply that 5-HT is acting directly on smooth muscle in portal vein. The antagonism by methysergide of low concentrations of 5-HT, and the antagonism by phentolamine of only high concentrations of this agonist suggest however, that 5-HT may be combining with two distinct sets of receptors, the activation of which depends on the concentration of drug employed.

In order to determine whether 5-HT and histamine were acting at a common receptor, an attempt was made to block the 5-HT response with diphenhydramine. This H_1 antagonist had appeared to reduce the response to histamine while having little effect on the response to noradrenaline. Diphenhydramine did, in fact, cause a depression of the response to 5-HT (Fig. 58). The depression of the response to this agonist occurred over all concentrations of agonist however, even though other results suggested that 5-HT may be acting at two different sets of receptors. These results, taken together with the very high concentration of the H_1 antagonists required to block the histamine and 5-HT responses, suggest that perhaps the H_1-antagonists are causing a somewhat non-selective reduction in the responses to these agonists.

Both the response to histamine and the response to 5-HT are much more sensitive to antagonism by phentolamine, than by other antagonists studied. Other α-adrenergic antagonists also blocked the histamine

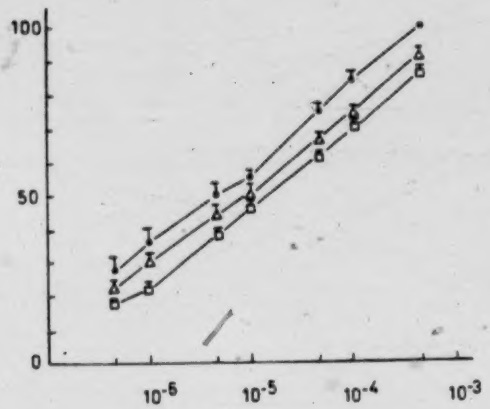


FIG. 58 The effect of diphenhydramine on the response to 5-HT. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : control dose-response curve to 5-HT.
Δ - Δ : 5-HT response after 10^{-6}M diphenhydramine.
□ - □ : 5-HT response after 5 x 10^{-6}M diphenhydramine.

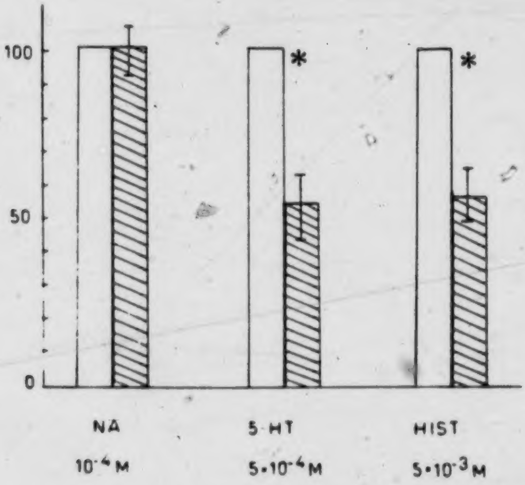


FIG. 59 Histogram illustrating the effect of desensitization to 5 x 10^{-4}M 5-HT on the maximum responses to noradrenaline (NA), histamine (HIST) and 5-HT. Per cent maximum response as ordinate. n = 8. Bars represent standard errors.

□ : control responses.

▨ : responses after induction of desensitization to 5-HT.

response as well as the noradrenaline response. Since both 5-HT and histamine appear to be acting directly on smooth muscle in this preparation, the evidence obtained so far implies that both these agonists and noradrenaline may be acting at the same receptor. As originally outlined in the Introduction, a number of approaches can be taken to determine whether or not this possibility is correct, and the results of some of these experiments are described in the following sections.

H. Further Attempts at Receptor Differentiation

Cross-desensitization should occur between the responses to noradrenaline, 5-HT and histamine, if these agonists are all acting at the same receptor. Desensitization to noradrenaline is very easily induced in portal vein, but it has a significant non-specific component, resulting in reduction of the acetylcholine response, as well as the responses to histamine and 5-HT. It was therefore decided to induce desensitization to 5-HT instead, and measure the effect of this procedure on the responses to noradrenaline and histamine. It was difficult to induce desensitization of the portal vein to this agonist, but when the response to 5-HT (5×10^{-4}M) was reduced to about 50% of the control value, the histamine response (5×10^{-3}M) was reduced by the same amount, while the noradrenaline response (10^{-4}M) appeared unaffected (Fig. 59).

At least two explanations of this phenomenon are possible. First, histamine and 5-HT could be acting at a common receptor, at which noradrenaline is not acting. The second, which is discussed more fully later, is that 5-HT and histamine are weak partial agonists at the α-adrenergic receptor in this preparation.

In order to examine the second possibility, dose-response curves to all three agonists were obtained on the same preparations, and plotted as a percentage of the maximum response to noradrenaline (Fig. 60). Even after the responses to histamine and 5-HT had been allowed to sensitize for 5 hours, the maximum response to histamine was only 75% and that to 5-HT only 85% of the maximum response to noradrenaline. In addition, there was approximately a one hundred-fold difference between the ED_{50} to noradrenaline ($2 \times 10^{-7}M$) and that to 5-HT ($10^{-5}M$), while the ED_{50} to histamine ($10^{-3}M$) occurred at almost one thousand times the concentration of that to noradrenaline.

These results indicate that if these agonists all act at the same receptor, noradrenaline would have to be considered a full agonist, and 5-HT and histamine partial agonists. In this case, using equi-effective concentrations of each agonist, in the ED_{50} range, would provide a truer measure of the effects of inducing desensitization than using the concentration of each agonist required for a maximum response.

Desensitization was again induced to 5-HT ($5 \times 10^{-4}M$), but this time, the effects were measured using equi-active doses of each agonist (Fig. 61). The responses to histamine ($2 \times 10^{-3}M$), 5-HT ($10^{-4}M$) and noradrenaline($2 \times 10^{-7}M$) were all reduced by this procedure, while the response to acetylcholine ($10^{-5}M$) was unaffected. The histamine response in this case appears less affected by this treatment but this may reflect a relatively greater increase in sensitivity to this concentration of histamine, rather than a reduced susceptibility to desensitization by 5-HT.

These results provide support for the hypothesis that all three agonists are acting at the same receptor. Ariens _et_ _al_. (1964) have provided evidence indicating that agents which act as partial agonists should also behave as partial antagonists of the responses to full

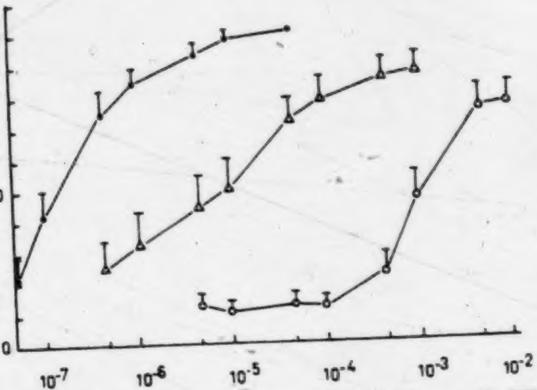


FIG. 60 Dose-response curves to noradrenaline, 5-HT and histamine, obtained on the same preparations 5 hours after the experiment was begun. All results expressed as a percentage of the maximum response to noradrenaline. Log molar concentration as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : dose-response curve to noradrenaline.
Δ - Δ : dose-response curve to 5-HT.
o - o : dose-response curve to histamine.

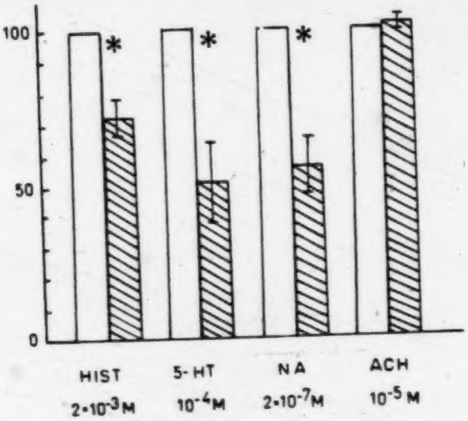


FIG. 61 Histogram illustrating the effect of desensitization to 5×10^{-4}M 5-HT on the responses to equiactive doses of histamine (HIST), noradrenaline (NA), 5-HT and acetylcholine (ACH). Per cent maximum response as ordinate. n = 7. Bars represent standard errors.

☐ : control responses.

▨ : responses after induction of desensitization to 5-HT.

agonists in the same preparation. In this situation then, it should be possible to antagonize the response to noradrenaline with either histamine or 5-HT. Initial attempts to do so were unsuccessful, and it was felt that the high concentrations of histamine and 5-HT being employed might be interfering with the uptake of noradrenaline. If this was occurring, then any antagonism of the response to noradrenaline would be concealed by the corresponding increase in sensitivity to this compound which is known to occur on inhibition of uptake (Trendelenburg, 1966). Thus it was decided first to block neuronal noradrenaline uptake with cocaine (3×10^{-5}M), and then to measure the effects of 5-HT and histamine on the noradrenaline response in the presence of cocaine. This procedure would be expected to minimize any further increase in sensitivity to noradrenaline due to the presence of histamine or 5-HT, allowing events occurring at the receptor to become more evident.

The results of antagonism of the noradrenaline response with histamine are shown in Fig. 62. Two concentrations of histamine, 10^{-3}M and 5×10^{-3}M, were used, but only the higher concentration had any significant effect on the response to noradrenaline. The nature of the antagonism produced by histamine is difficult to describe however, although it does not appear to be a simple competitive type of antagonism. 5-HT, in concentrations of 10^{-4}M and 5×10^{-4}M, had no significant effect on the response to noradrenaline (Fig. 63), even in the presence of cocaine.

I. Receptor Protection Experiments

In experiments involving receptor protection, each agonist, histamine, noradrenaline and 5-HT, was used as a protecting agent, in an attempt to prevent blockade of the responses to each of the other agonists

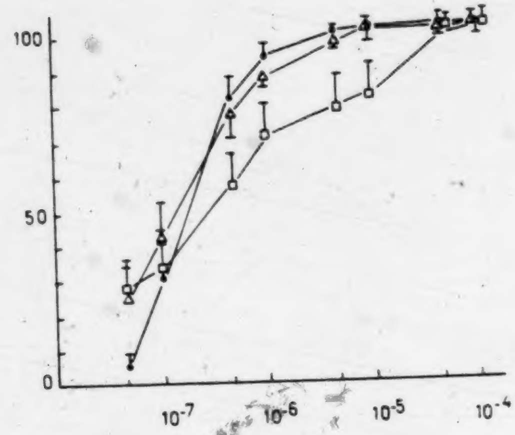


FIG. 62 The effect of histamine on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 9. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-3}M histamine.
□ - □ : noradrenaline response after 5 x 10^{-3}M histamine.

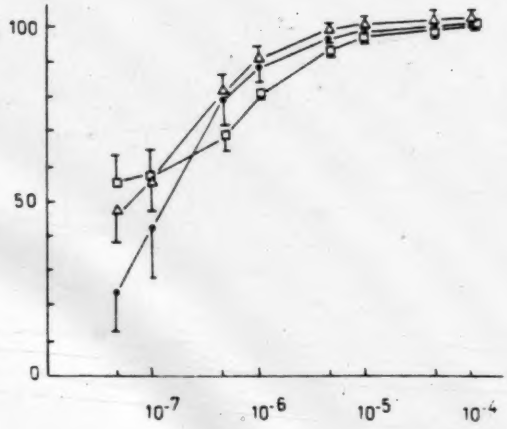


FIG. 63 The effect of 5-HT on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 10^{-4}M 5-HT.
□ - □ : noradrenaline response after 5 x 10^{-4}M 5-HT.

with the irreversible antagonist, phenoxybenzamine (POB). Phentolamine was also used as a protecting agent of the responses to all three agonists, while attempts were made to protect the histamine response with diphenhydramine. Concentrations of each agonist required to produce the maximum response were used for protection, while the concentration of phentolamine used was 5 x 10^{-7}M and that of diphenhydramine 5 x 10^{-6}M.

Initially, dose-response curves to each agonist in the presence of POB alone were obtained. POB (5 x 10^{-9}M) depressed the maximum response to noradrenaline to 60% of the control value (Fig. 64). This response was established within 15 minutes after the addition of the antagonist to the bath, and appeared irreversible, since washing for up to 3 hours in the presence of sodium thiosulfate produced no reversal of the blockade.

This same concentration of POB (5 x 10^{-9}M) also produced blockade of the responses to histamine and 5-HT. The histamine response was reduced to about 50% of the initial maximum response (Fig. 65). When the histamine response in the presence of POB is plotted on the same graph as the histamine response in untreated paired controls obtained at the same time, it can be seen that the true blockade produced is actually greater than 50% (Fig. 66). The 5-HT response after exposure to POB is reduced to less than 70% of the control maximum response (Fig. 67). Although untreated paired controls were not measured, it is likely that this is also an underestimation of the amount of blockade produced, since there is significant sensitization of the tissue to 5-HT also. The response to concentrations of 5-HT below 5 x 10^{-6}M were not blocked by this concentration of POB, so results obtained at these

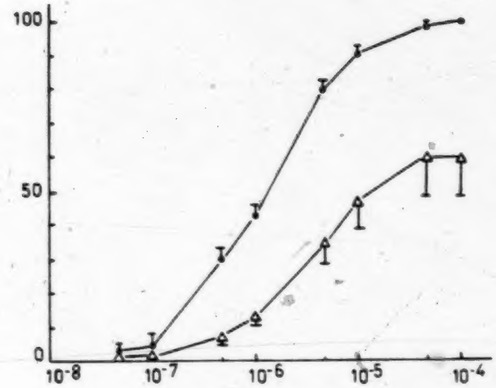


FIG. 64 The effect of POB on the response to noradrenaline. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 4. Bars represent standard errors.

● - ● : control dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after 5 x 10^{-9}M POB (3 minutes).

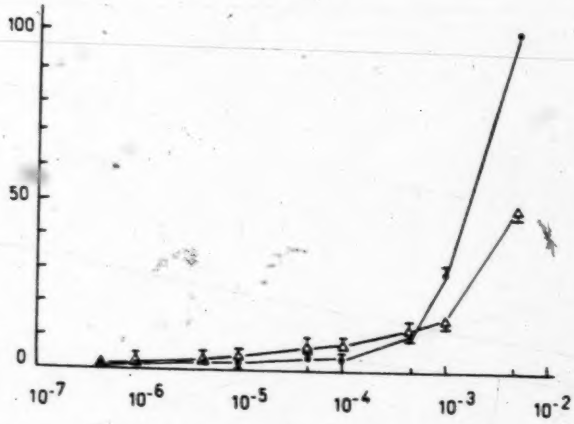


FIG. 65 The effect of POB on the response to histamine. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 22. Bars represent standard errors.

● - ● : initial dose-response curve to histamine.
Δ - Δ : histamine response after 5 x 10^{-9}M POB (3 minutes).

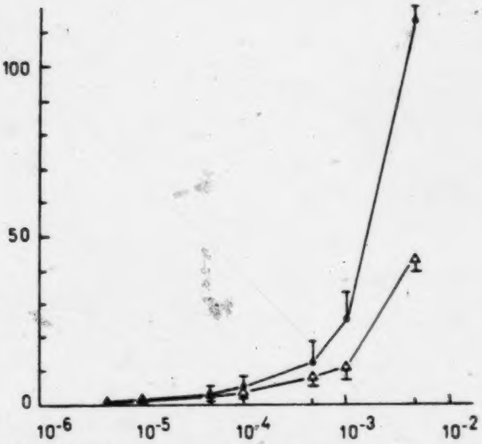


FIG. 66 The histamine response obtained on paired unblocked control tissues, plotted on the same graph as the histamine response after blockade with POB. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● – ● : histamine response of paired unblocked controls.
Δ – Δ : histamine response after 5 x 10^{-9}M POB (3 minutes).

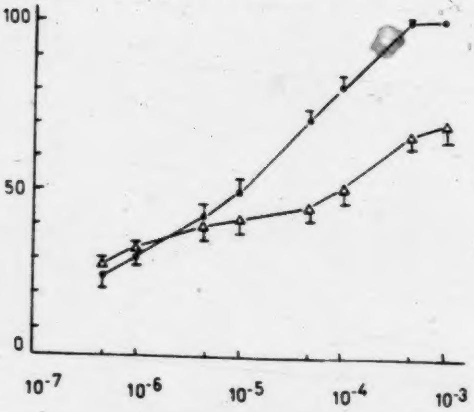


FIG. 67 The effect of POB on the response to 5-HT. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 14. Bars represent standard errors.

● – ● : control dose-response curve to 5-HT.
Δ – Δ : 5-HT response after 5 x 10^{-9}M POB (3 minutes).

concentrations were omitted from the graphs of results of protection experiments.

The results of self-protection of the responses to each agonist were next obtained. The noradrenaline response was fully protected against POB blockade with 10^{-4}M noradrenaline (Fig. 68). 5-HT (5×10^{-4}M) provided partial protection of the response to 5-HT (Fig. 69). The histamine response was also partially protected against POB blockade with 5×10^{-3}M histamine (Fig. 70).

None of the other agents used to protect the noradrenaline response gave full protection. Phentolamine (5×10^{-7}M) gave almost complete protection however, even when used in a concentration somewhat lower than is usual for protection experiments (Fig. 71). 5-HT (5×10^{-4}M) also gave significant protection of the noradrenaline response (Fig. 72), while histamine (5×10^{-3}M) provided only partial protection (Fig. 73).

The histamine response was fully protected by noradrenaline (10^{-4}M), although this concentration also caused significant desensitization of the response to histamine, which was not completely reversed even after a one hour wash period (Fig. 74). Phentolamine (5×10^{-7}M) protected the histamine response partially but significantly against POB blockade (Fig. 75), while 5-HT (5×10^{-4}M) provided complete protection (Fig. 76). Since diphenhydramine had been shown earlier to reduce the histamine response, and the suggestion was made that this antagonism was non-selective (see Section F), it was of interest to determine whether or not this antagonist could protect the histamine response. Results are shown in Fig. 77; diphenhydramine (5×10^{-6}M) does not appear to protect the histamine response against

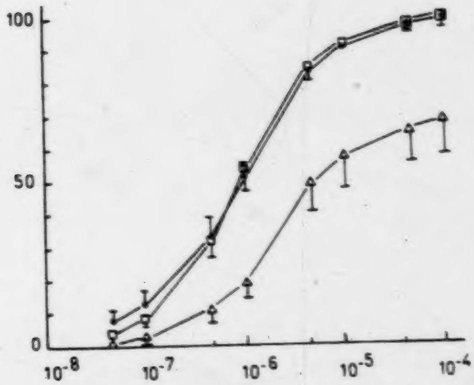


FIG. 68 The effect of protection with 10^{-4}M noradrenaline on the blockade of the noradrenaline response by POB. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : initial dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after POB alone ('control blocked').
□ - □ : noradrenaline response after POB plus noradrenaline ('protected blocked').

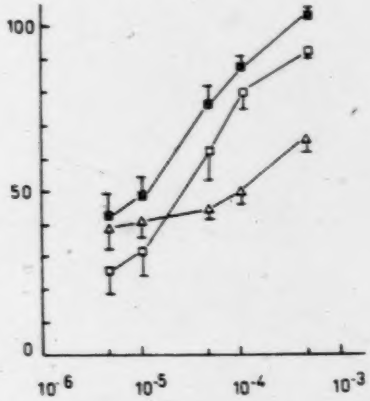


FIG. 69 The effect of protection with 5 x 10^{-4}M 5-HT on the blockade of the 5-HT response by POB. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

■ - ■ : 5-HT response after 5-HT alone ('control').
Δ - Δ : 5-HT response after POB alone ('control blocked').
□ - □ : 5-HT response after POB plus 5-HT ('protected blocked').

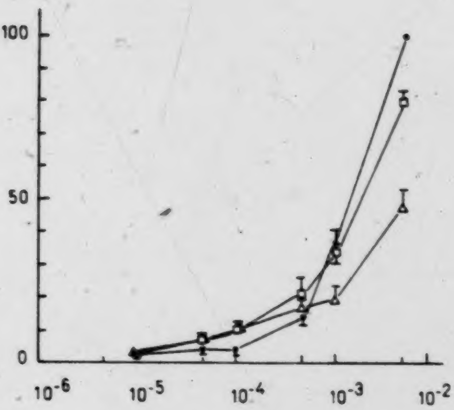


FIG. 70 The effect of protection with 5×10^{-3}M histamine on the blockade of the histamine response by POB. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : initial dose-response curve to histamine.
Δ - Δ : histamine response after POB alone ('control blocked').
□ - □ : histamine response after POB plus histamine ('protected blocked').

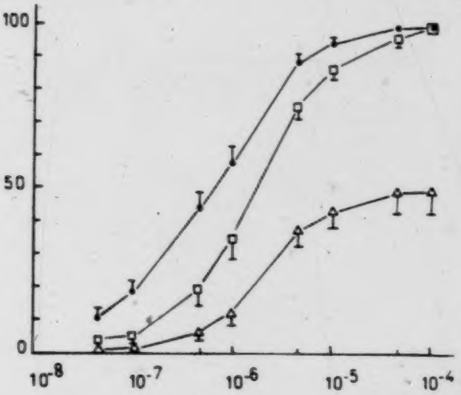


FIG. 71 The effect of protection with 5×10^{-7}M phentolamine on the blockade of the noradrenaline response by POB. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : initial dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after POB alone ('control blocked').
□ - □ : noradrenaline response after POB plus phentolamine ('protected blocked').

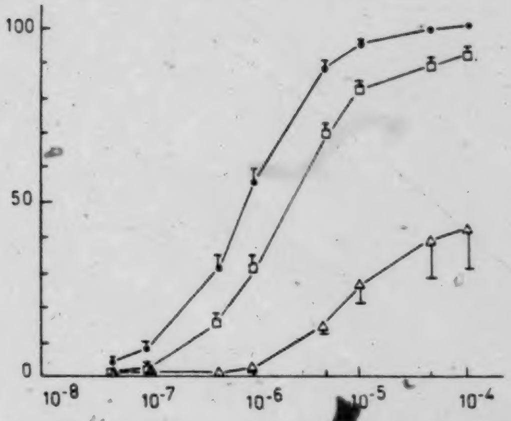


FIG. 72 The effect of protection with 5 x 10^{-4}M 5-HT on the blockade of the noradrenaline response by POB. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : initial dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after POB alone ('control blocked').
□ - □ : noradrenaline response after POB plus 5-HT ('protected blocked').

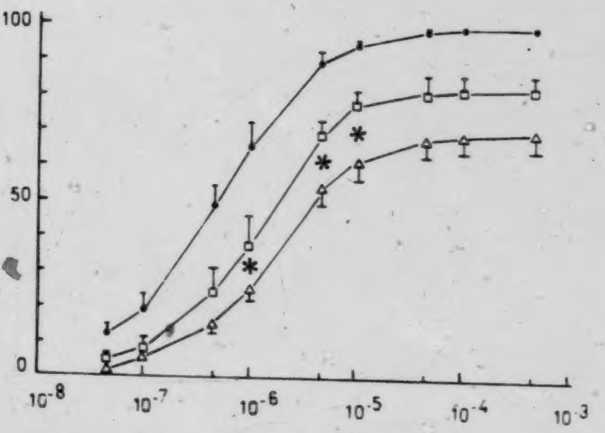


FIG. 73 The effect of protection with 5 x 10^{-3}M histamine on the blockade of the noradrenaline response by POB. Log molar concentration of noradrenaline as abscissa, per cent maximum response as ordinate. n = 7. Bars represent standard errors.

● - ● : initial dose-response curve to noradrenaline.
Δ - Δ : noradrenaline response after POB alone ('control blocked').
□ - □ : noradrenaline response after POB plus histamine ('protected blocked').

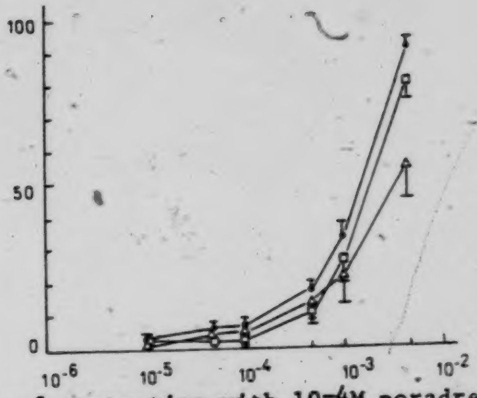


FIG. 74 The effect of protection with 10^{-4}M noradrenaline on the blockade of the histamine response by POB. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 10. Bars represent standard error.

● - ● : histamine response after 10^{-4}M noradrenaline alone. ('control').

Δ - Δ : histamine response after POB alone ('control blocked').

□ - □ : histamine response after POB plus noradrenaline ('protected blocked').

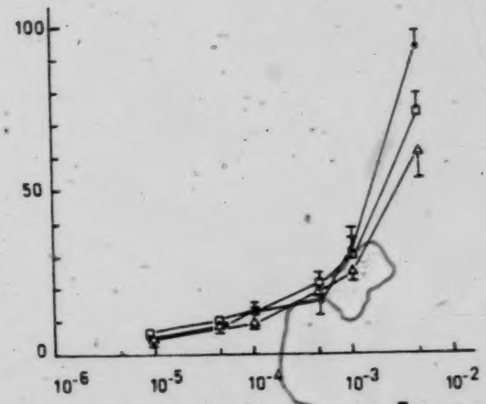


FIG. 75 The effect of protection with 5 x 10^{-7}M phentolamine on the blockade of the histamine response by POB. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 8. Bars represent standard errors.

● - ● : histamine response after phentolamine alone ('control').

Δ - Δ : histamine response after POB alone ('control blocked').

□ - □ : histamine response after POB plus phentolamine ('protected blocked').

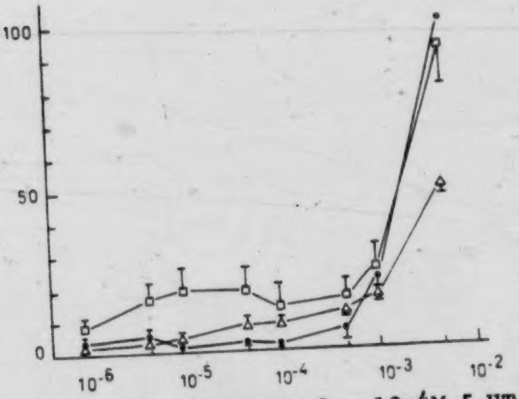


FIG. 76 The effect of protection with 5 x 10^{-4}M 5-HT on the blockade of the histamine response by POB. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● – ● : histamine response after 5-HT alone ('control').
Δ – Δ : histamine response after POB alone ('control blocked').
□ – □ : histamine response after POB plus 5-HT ('protected blocked').

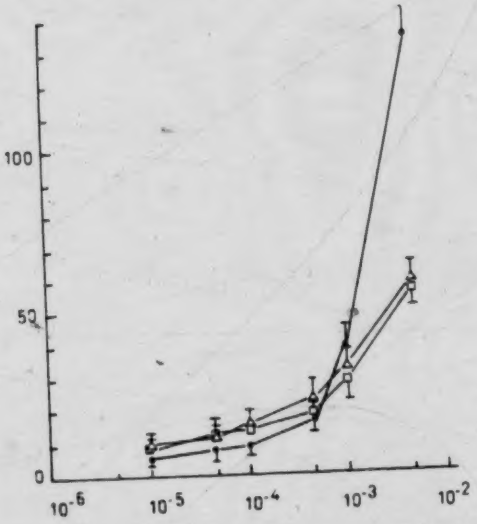


FIG. 77 The effect of protection with 5 x 10^{-6}M diphenhydramine on the blockade of the histamine response by POB. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 7. Bars represent standard errors.

● – ● : histamine response after diphenhydramine alone ('control').
Δ – Δ : histamine response after POB alone ('control blocked').
□ – □ : histamine response after POB plus diphenhydramine ('protected blocked').

POB blockade, even in a concentration sufficient to reduce the histamine response to 40% of the control value.

The response to 5-HT, when protected with noradrenaline (10^{-4}M), is somewhat unusual (Fig. 78). In tissues treated with POB, the 5-HT response after exposure to noradrenaline is increased over the 5-HT response in unblocked tissues after exposure to noradrenaline. The reason for this phenomenon is not clear at this time, but certainly noradrenaline does protect the response to 5-HT. Phentolamine (5×10^{-7}M) also protects the 5-HT response against blockade with POB (Fig. 79), appearing to protect the response at high concentrations of 5-HT somewhat better than the lower concentrations. The response to 5-HT is not protected significantly by histamine (5×10^{-3}M) (Fig. 80).

The technique of receptor protection has come under a good deal of criticism, mainly as a result of the very high concentrations of protecting agents commonly used, which may provide non-specific protection of sites other than the one through which the agent has its action (Waud, 1962). In the experiments described in this section the concentration of phentolamine used for protection was deliberately kept low (5×10^{-7}M) in order to avoid this problem. It was not possible to reduce the concentrations of agonists used for protection however, because the concentrations of 5-HT and histamine necessary to produce the maximum response were very high, and these were felt to be the minimum concentrations necessary for significant protection to occur. Therefore, to determine whether the concentrations of agonists used as protective agents were providing non-specific protection, attempts were made to protect the acetylcholine response against POB blockade with each of them. Although it was hoped that this method might give some

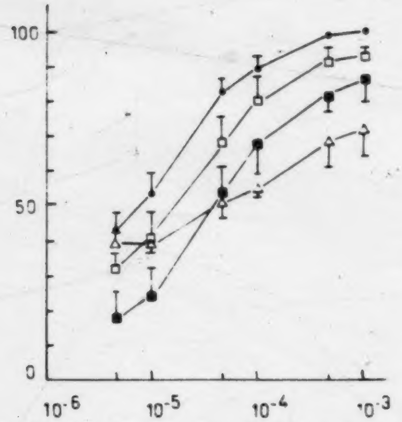


FIG. 78 The effect of protection with 10^{-4}M noradrenaline on the blockade of the 5-HT response by POB. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : initial dose-response curve to 5-HT.
■ - ■ : 5-HT response after noradrenaline alone ('control').
Δ - Δ : 5-HT response after POB alone ('control blocked').
□ - □ : 5-HT response after POB plus noradrenaline ('protected blocked').

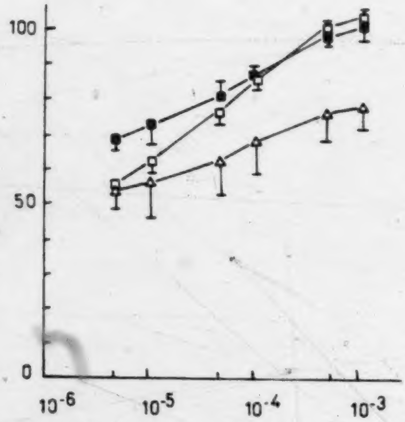


FIG. 79 The effect of protection with 5 x 10^{-7}M phentolamine on the blockade of the 5-HT response by POB. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 5. Bars represent standard errors.

■ - ■ : 5-HT response after phentolamine alone ('control').
Δ - Δ : 5-HT response after POB alone ('control blocked').
□ - □ : 5-HT response after POB plus phentolamine ('protected blocked').

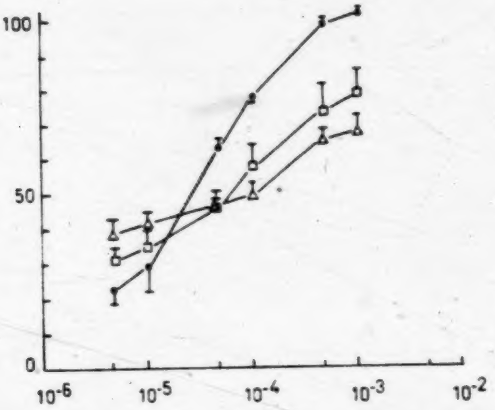


FIG. 80 The effect of protection with 5 x 10^{-3}M histamine on the blockade of the 5-HT response by POB. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● – ● : 5-HT response after histamine alone ('control').
Δ – Δ : 5-HT response after POB alone ('control blocked').
□ – □ : 5-HT response after POB plus histamine ('protected blocked').

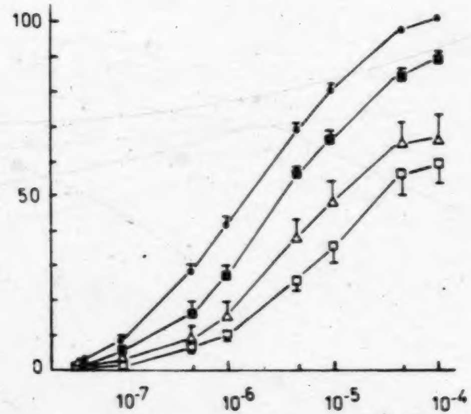


FIG. 81 The effect of protection with 10^{-4}M noradrenaline on the blockade of the acetylcholine response by POB. Log molar concentration of acetylcholine as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● – ● : initial dose-response curve to acetylcholine.
■ – ■ : acetylcholine response after noradrenaline alone ('control').
Δ – Δ : acetylcholine response after POB alone ('control blocked').
□ – □ : acetylcholine response after POB plus noradrenaline ('protected blocked').

indication of the specificity of the protection afforded by each of these agonists, this information was limited by the fact that the concentration of POB necessary to reduce the acetylcholine response to 60% of the control value was in the range of 5 x 10^{-7}M to 10^{-6}M. This is 100-200 times the concentration of POB necessary to block the noradrenaline response to the same extent, and concentrations of agonist which protect against blockade by the low concentration of POB might not be expected to have an effect against the high concentrations.

Noradrenaline (10^{-4}M) provided no protection of the acetylcholine response against blockade by 10^{-6}M POB (Fig. 81). The response to Ach, like the responses to other agonists tested, was desensitized by noradrenaline, and the desensitization was not fully reversed by washing the tissues for one hour. Unlike the responses to the other agonists however, when the Ach response is blocked with POB in the presence of noradrenaline, this response is reduced to less than the response obtained after blockade with POB alone.

When the response to Ach is protected against 5 x 10^{-7}M POB with 5-HT (5 x 10^{-4}M), however, 5-HT is found to provide almost complete protection of the Ach response (Fig. 82). This occurs even though 5-HT has no apparent action through cholinergic receptors, the response to 5-HT being unaffected by 10^{-7}M atropine (Fig. 83).

The Ach response is not significantly protected against blockade by 10^{-6}M POB when histamine (5 x 10^{-3}M) is used as the protecting agent (Fig. 84).

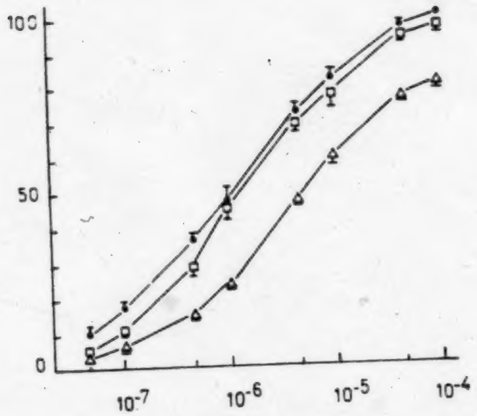


FIG. 82 The effect of protection with 5 x 10^{-4}M 5-HT on the blockade of the acetylcholine response by POB. Log molar concentration of acetylcholine as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

● - ● : acetylcholine response after 5-HT alone ('control').
Δ - Δ : acetylcholine response after POB alone ('control blocked').
□ - □ : acetylcholine response after POB plus 5-HT ('protected blocked').

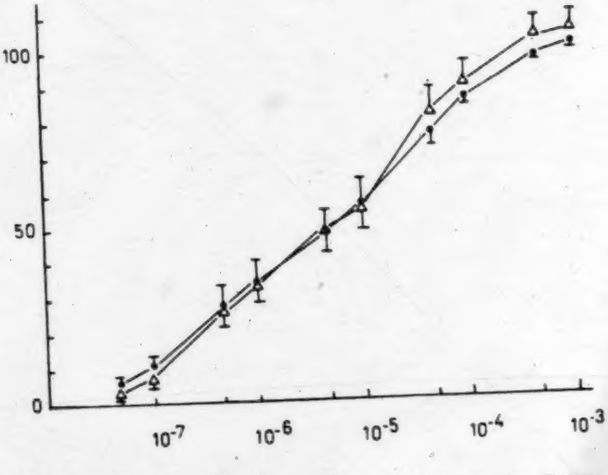


FIG. 83 The effect of atropine on the response to 5-HT. Log molar concentration of 5-HT as abscissa, per cent maximum response as ordinate. n = 5. Bars represent standard errors.

● - ● : control dose-response curve to 5-HT.
Δ - Δ : 5-HT response after 10^{-7}M atropine.

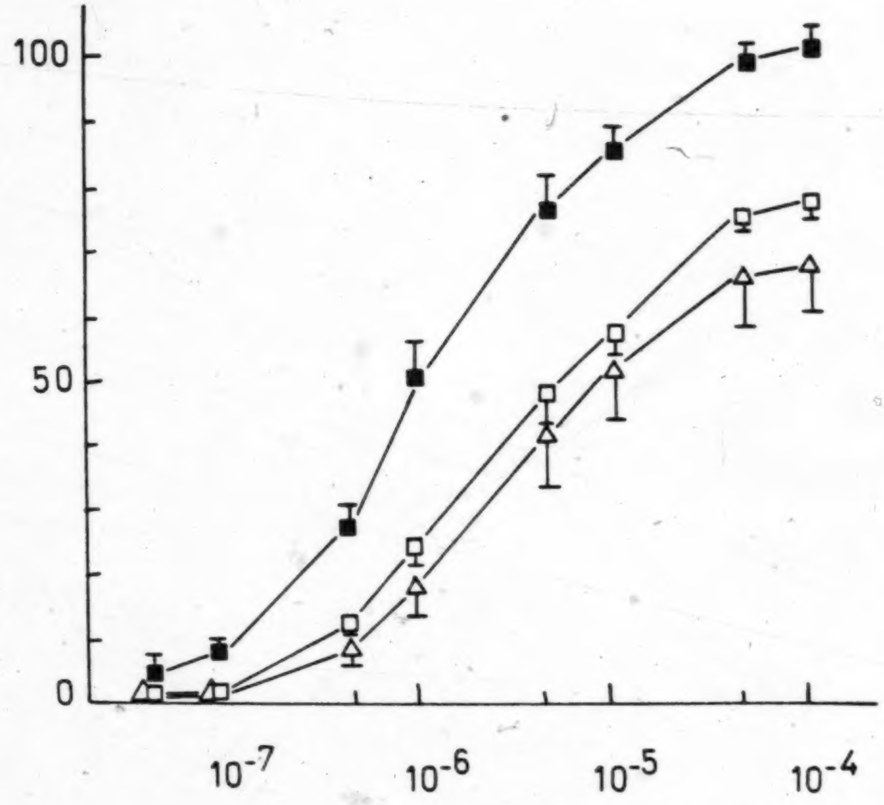


FIG. 84 The effect of protection with 5 x 10^{-3}M histamine on the blockade of the acetylcholine response by POB. Log molar concentration of acetylcholine as abscissa, per cent maximum response as ordinate. n = 6. Bars represent standard errors.

■ - ■ : acetylcholine response after histamine alone ('control').
Δ - Δ : acetylcholine response after POB alone ('control blocked').
□ - □ : acetylcholine response after POB plus histamine ('protected blocked').

II THE UPTAKE AND EFFLUX OF [^{3}H]-NORADRENALINE

The experiments described in this section were conducted for two reasons. The first was to determine whether the conclusions concerning the release of noradrenaline by histamine and 5-HT in isolated tissues were supported by direct measurement of [^{3}H]-noradrenaline efflux. The second was to provide some information concerning the mechanism of the sensitization of rabbit portal vein to histamine; this is discussed in Section III.

The uptake of [^{3}H]-noradrenaline was measured for 60 minutes, in the presence and absence of cocaine (Fig. 85). The T/M ratio in the absence of cocaine reaches approximately 4.5 g/ml after 60 minutes, which indicates that accumulation of [^{3}H]-noradrenaline into the tissue is taking place. Uptake of noradrenaline in the presence of cocaine is reduced but not abolished, suggesting that both neuronal uptake (cocaine sensitive), and non-neuronal uptake (cocaine insensitive) occurs in this tissue.

The efflux of [^{3}H]-noradrenaline was also followed, and appeared to consist of at least two major phases (Fig. 86). Loss of tracer from the initial fast compartment was essentially complete within 20 to 30 minutes of the start of the efflux period. The slower component had a much longer half-time and was the only detectable compartment responsible for tracer loss by 70 minutes, when the addition of drugs took place. This slow component is believed to result from the efflux of noradrenaline from sympathetic nerve terminals (Paton, 1973).

When tyramine (10^{-4}M), 5-HT (5×10^{-4}M) or histamine (5×10^{-3}M) are placed in the bathing medium, there is an immediate significant increase in the efflux coefficient of [^{3}H]-noradrenaline (Fig. 86),

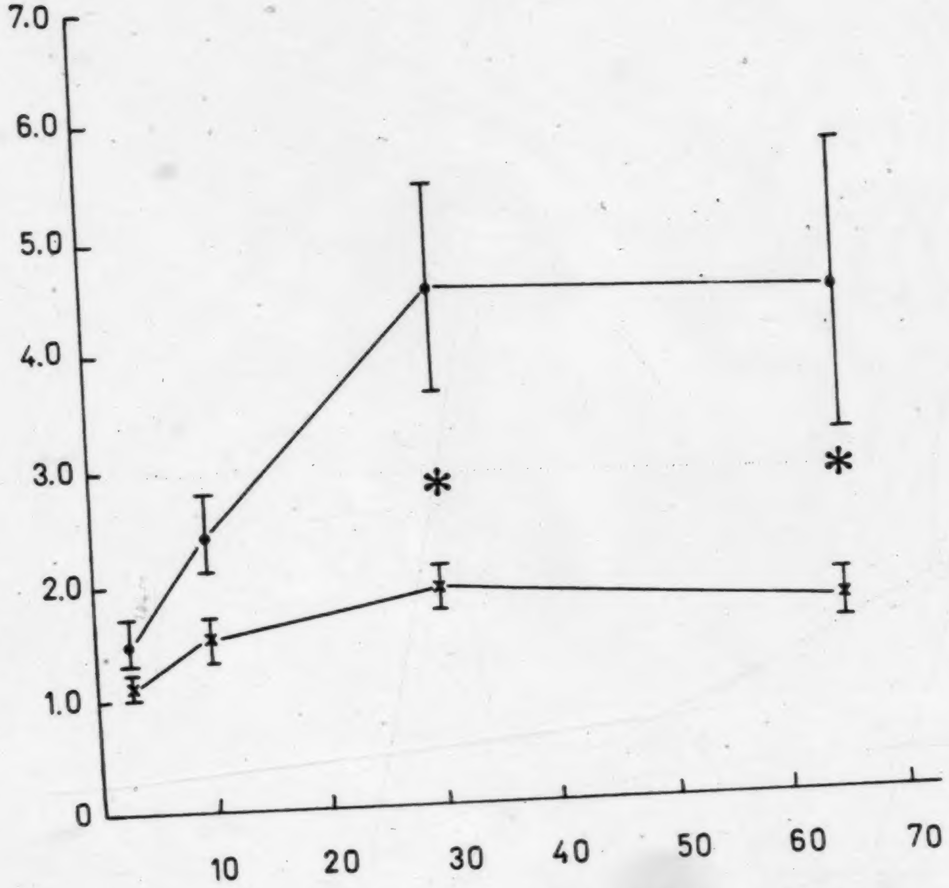


FIG. 85 The uptake of [^{3}H]-noradrenaline. T/M ratio (g/ml) as ordinate, time in minutes as abscissa n = 4. Bars represent standard errors.

● - ● : uptake in untreated tissues.
x - x : uptake in the presence of 3 x 10^{-5}M cocaine.

Significance obtained using the t-test for paired data.

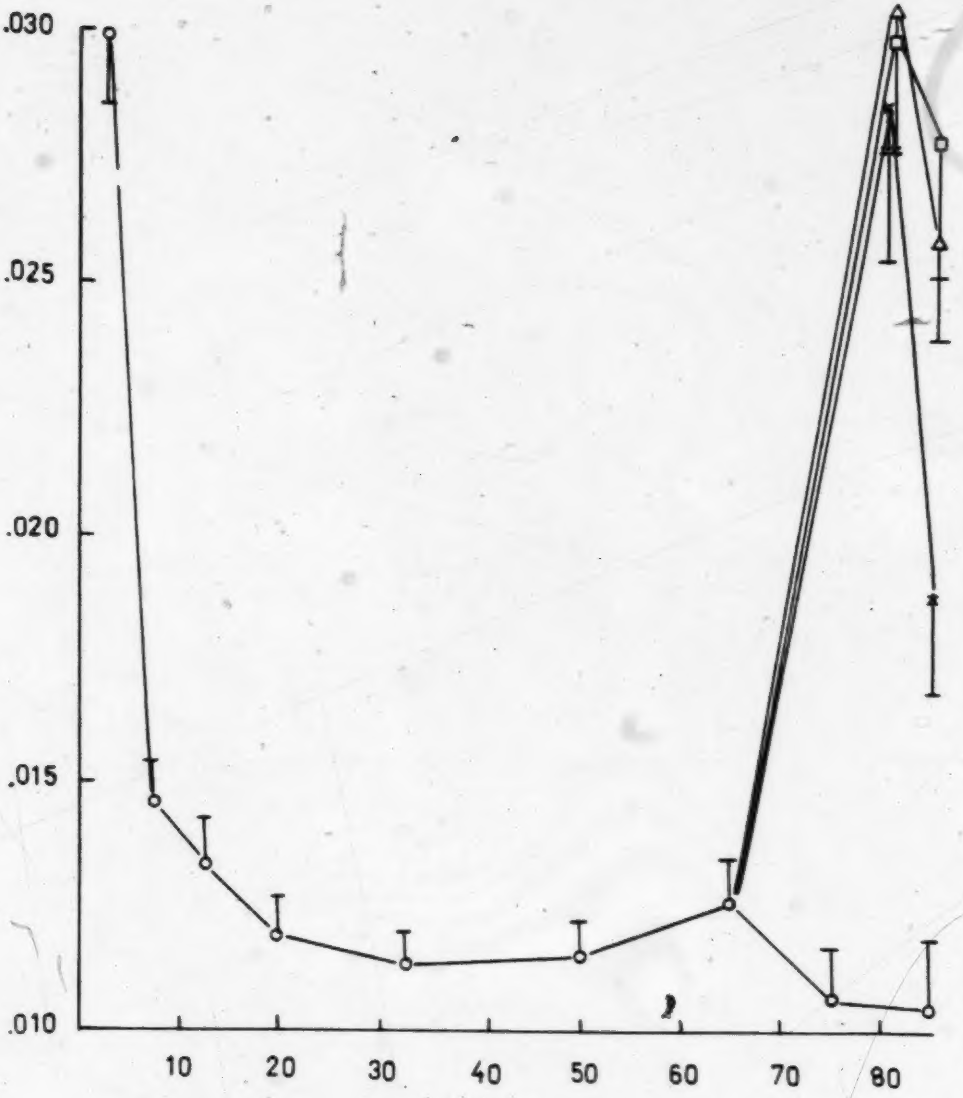


FIG. 86 [^{3}H]-noradrenaline efflux under control conditions. Efflux coefficient (E_{CO}) as ordinate, time in minutes as abscissa. Time 0 to 65 minutes, n - 23. Bars represent standard errors.

o - o : efflux from untreated tissues (n = 6).
x - x : efflux from tissues treated with 5 x 10^{-3}M histamine (n = 6).
Δ - Δ : efflux from tissues treated with 5 x 10^{-4}M 5-HT (n = 6).
□ - □ : efflux from tissues treated with 10^{-4}M tyramine (n = 5).

which appears to be of the same magnitude for each agent. Phenylephrine has been shown to cause an efflux of [^{3}H]-noradrenaline, which is unrelated to its major mode of action. This occurred when efflux took place in the presence of monoamine oxidase and catechol-O-methyl transferase inhibitors, and was much reduced in the absence of these agents (D.M. Paton, personal communication). In order to determine whether a similar mechanism accounted for the release of [^{3}H]-noradrenaline by 5-HT and histamine in this preparation, efflux was monitored in the absence of pargyline and tropolone. The results are shown in Fig. 87. The overall efflux coefficients are lower than those measured in tissues in the presence of inhibitors. However, 5-HT and histamine still cause a significant increase in efflux, and this increase is not different from that caused by tyramine.

Thus the possibility again arose that histamine and 5-HT are acting by noradrenaline release, but that for some reason, treatment of isolated preparations with cocaine or 6-OHDA resulted in the abolition of the release by tyramine but not by 5-HT and histamine. In order to examine this possibility further, preparations were treated with cocaine and 6-OHDA before the effects of tyramine, histamine or 5-HT on efflux were measured.

Pre-treatment of preparations with cocaine before the addition of agonists resulted in a significant decrease compared to control in the [^{3}H]-noradrenaline efflux measured in the presence of all the agonists (Figs. 88,89). Yet earlier results had indicated that exposure of isolated preparations to cocaine resulted in a significant decrease in the contractile response to tyramine but not histamine (Fig. 31) or 5-HT.

The 6-OHDA-treated preparations were treated in a similar manner to

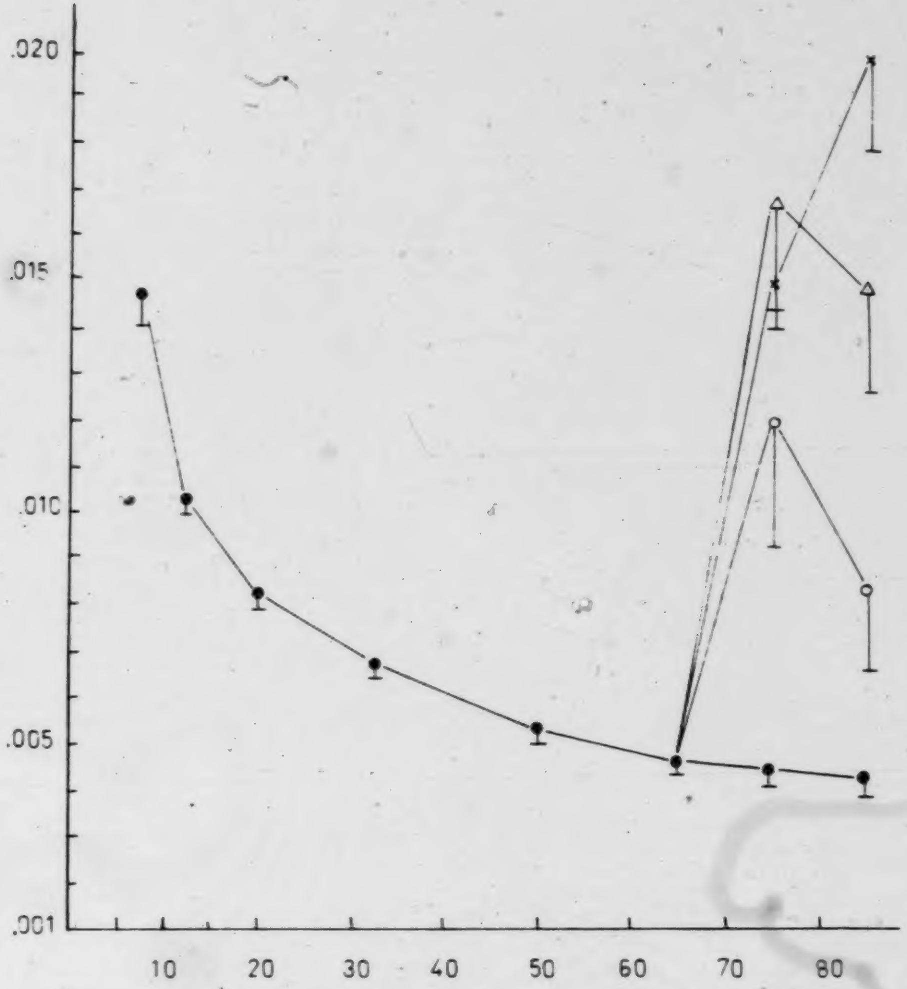


FIG. 87 [^{3}H]-noradrenaline efflux measured in the absence of monoamine oxidase and catechol-O-methyl transferase inhibitors. Efflux coefficient (E_{co}) as ordinate, time in minutes as abscissa. Time 0 to 65 minutes, n = 22. Bars represent standard errors.

● - ● : efflux from untreated tissues (n = 6).
x - x : efflux from tissues treated with 5 x 10^{-3}M histamine (n = 6).
Δ - Δ : efflux from tissues treated with 5 x 10^{-4}M 5-HT (n = 6).
o - o : efflux from tissues treated with 10^{-4}M tyramine (n - 5).

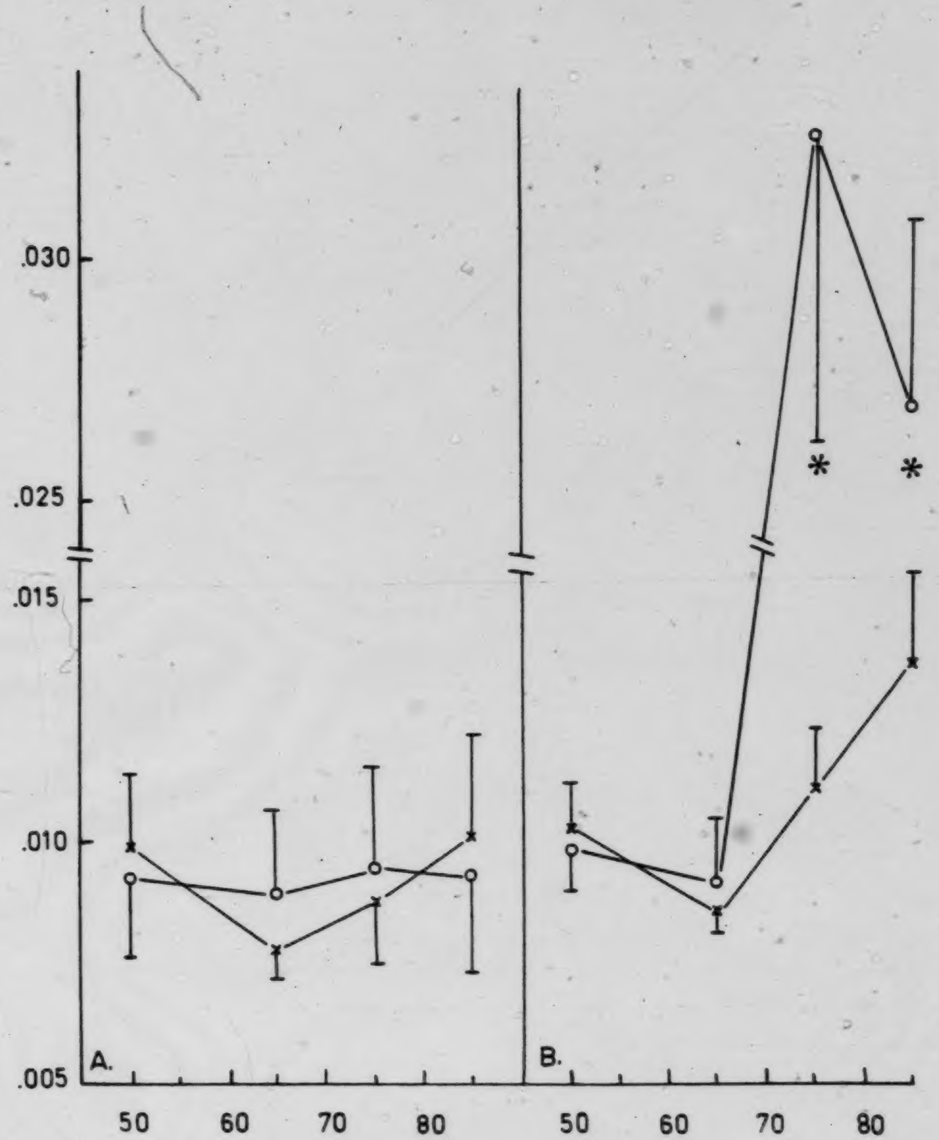


FIG. 88 The effect of drugs on the efflux of [^{3}H]-noradrenaline from untreated tissues and tissues treated with cocaine. Efflux coefficient (E_{co}) as ordinate, time in minutes as abscissa. n = 4. Bars represent standard errors.

A. Efflux from control tissues
B. Efflux from tissues treated with 5 x 10^{-3}M histamine.

o – o : untreated tissues.
x – x : tissues treated with 3 x 10^{-5}M cocaine.

Significance obtained using the t-test for paired data.

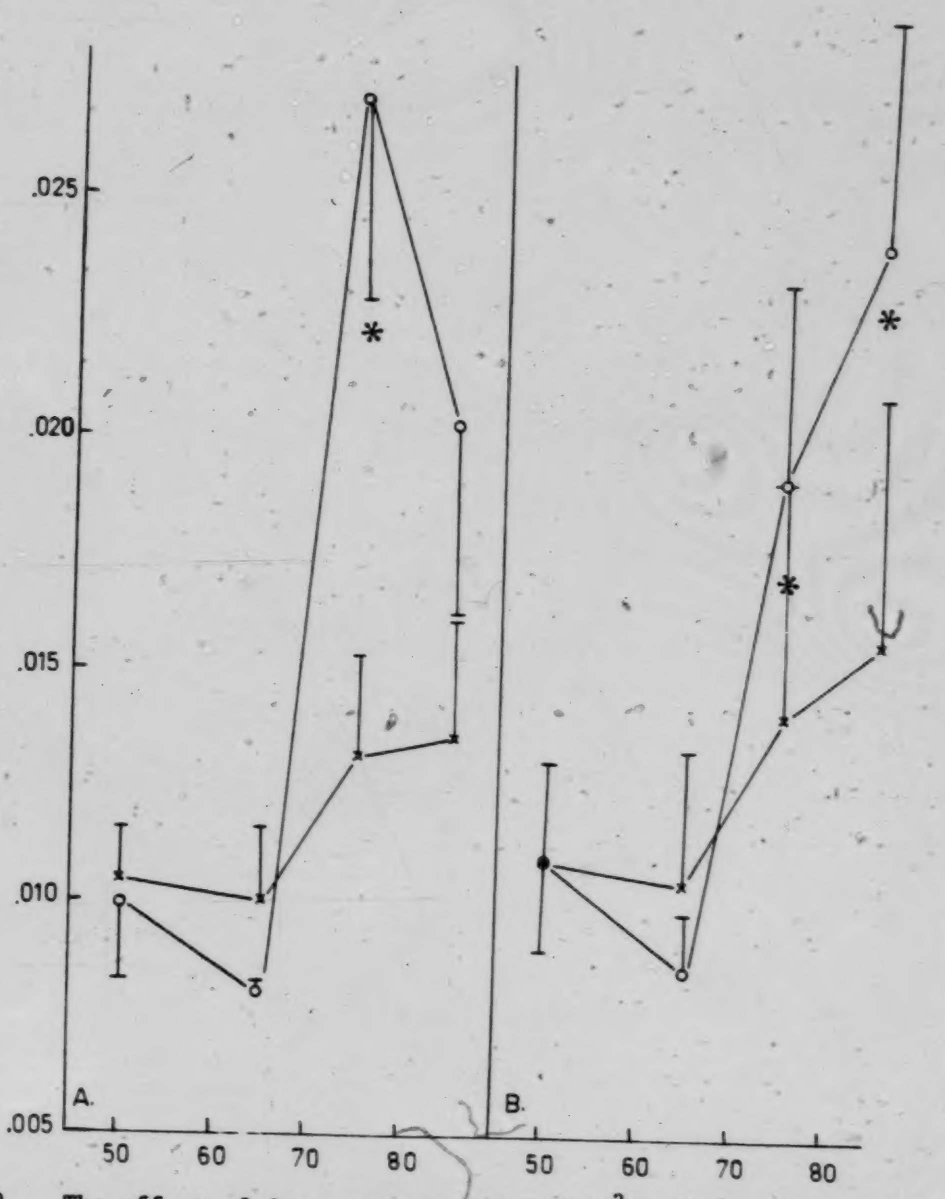


FIG. 89 The effect of drugs on the efflux of [^{3}H]-noradrenaline from untreated tissues and tissues treated with cocaine. Efflux coefficient as ordinate, time in minutes as abscissa.

A. Efflux from tissues treated with 5 x 10^{-4}M 5-HT.
B. Efflux from tissues treated with 10^{-5}M tyramine.

o - o : untreated tissues.
x - x : tissues treated with 3 x 10^{-5}M cocaine.

Significance obtained using the t-test for paired data.

the isolated preparations - after a 15 minute exposure to 6-OHDA the tissues were washed for 2 hours before being placed in a solution containing the drug to be tested. Results of efflux from control tissues are shown in Fig. 90a. Efflux from tissues treated with 6-OHDA was lower than that in control tissues, but was not abolished. The effect of 6-OHDA treatment on efflux in the presence of drugs is shown in Figs. 90b and 91 - in all cases, after 6-OHDA treatment, there was no significant increase in efflux as occurred in control preparations when tissues were treated with histamine, 5-HT or tyramine.

This treatment results in almost abolishing the pharmacological response to tyramine, but has no selective effect on the response to 5-HT or histamine (see Section F). Thus although both these agonists appear to cause some release of [^{3}H]-noradrenaline, this does not seem to be the means by which they have their main action. The possibility that release of noradrenaline by these agonists might be involved in the sensitization seen to them is discussed in the next section.

III THE MECHANISM OF SENSITIZATION

The sensitization of rabbit portal vein to histamine was first mentioned in Section I-A. The sensitization develops with time, and seems specific for histamine, since it was not seen to occur to noradrenaline and acetylcholine. The amount of sensitization seen was reduced in tissues taken from rabbits pre-treated with reserpine; in Fig. 92, the dose-response curves to histamine obtained after 5 hours from reserpinized tissues and control tissues are plotted on the same graph for comparison. These results led to the conclusion that the sensitization was specific for histamine, and might have an adrenergic

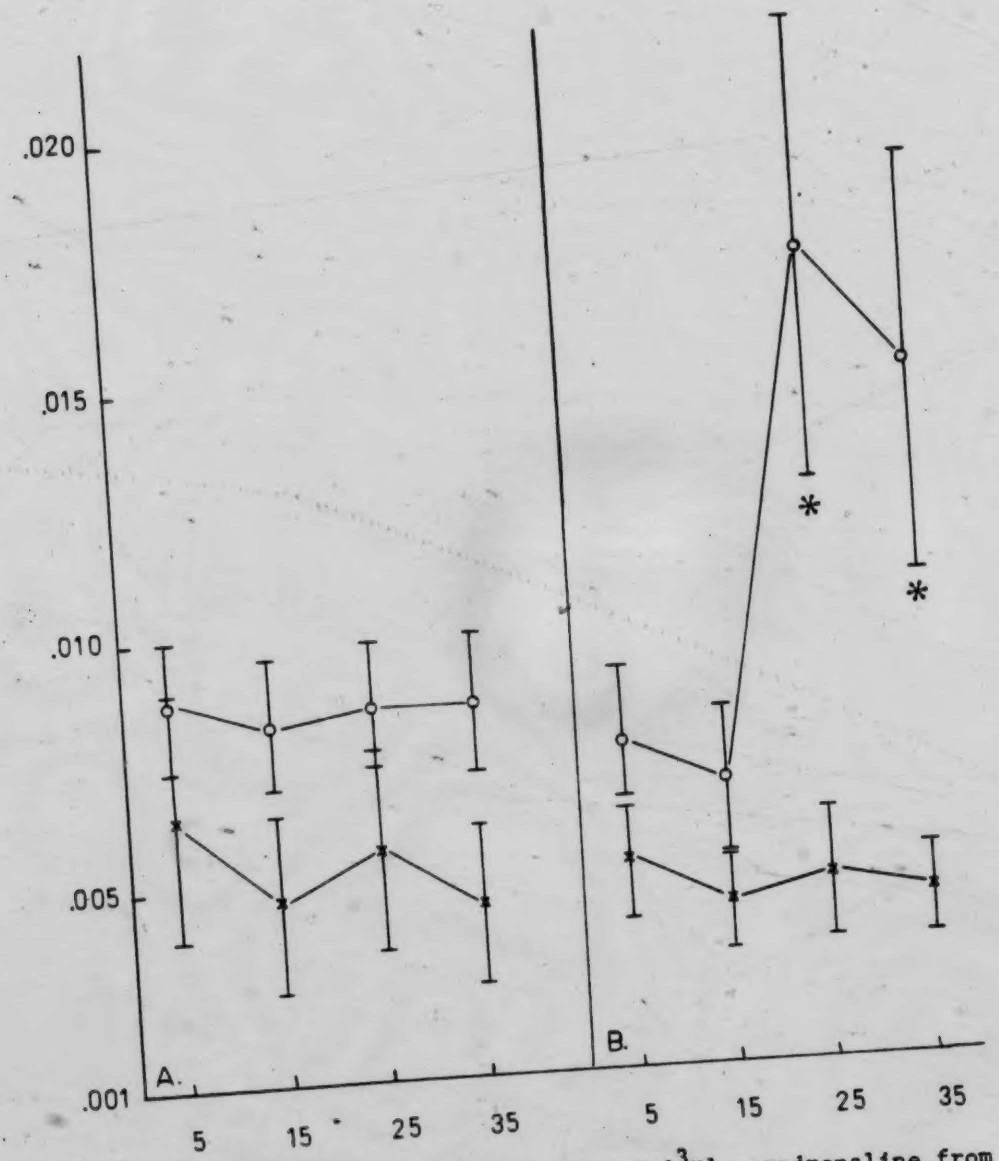


FIG. 90 The effect of drugs on the efflux of [^{3}H]-noradrenaline from untreated tissues and tissues treated with 6-OHDA. Efflux coefficient (E_{CO}) as ordinate, time in minutes as abscissa. n = 4. Bars represent standard errors.

A. Efflux from control tissues.
B. Efflux from tissues treated with 5 x 10^{-3}M histamine.

o - o : untreated tissues.
x - x : tissues treated with 250 µg/ml 6-OHDA.

Significance obtained using the t-test for paired data.

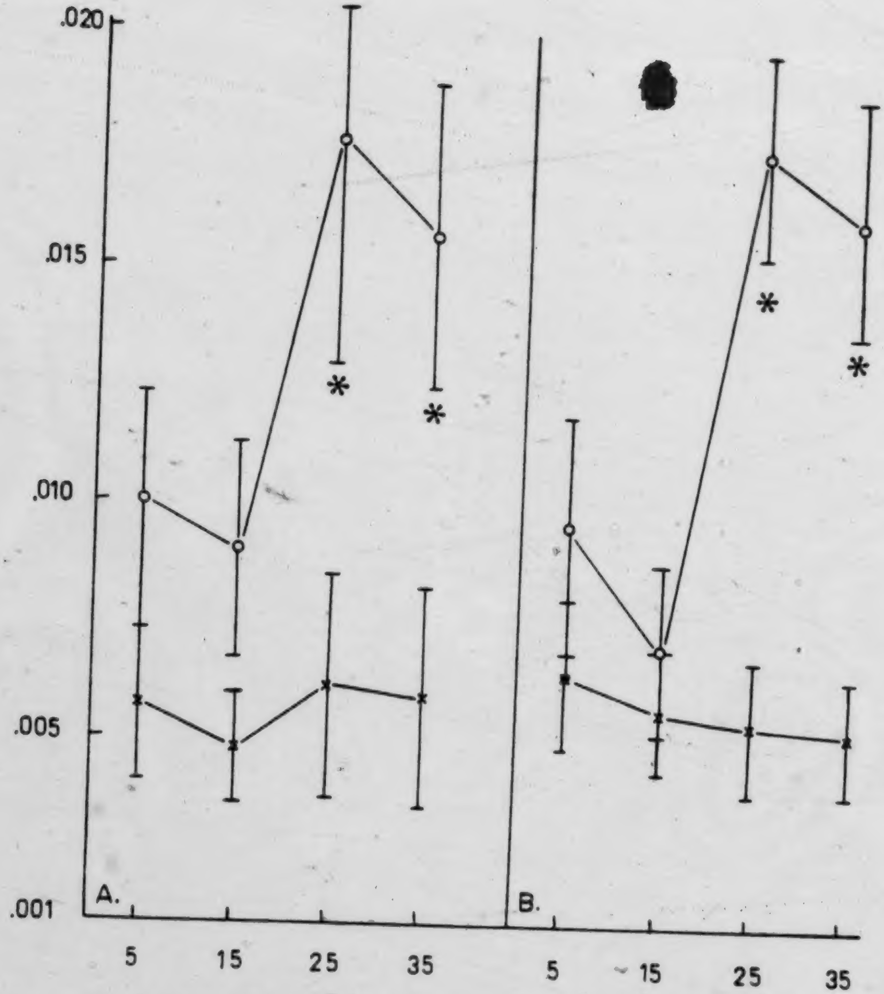


FIG. 91 The effect of drugs on the efflux of [^{3}H]-noradrenaline from untreated tissues and tissues treated with 6-OHDA. Efflux coefficient as ordinate, time in minutes as abscissa.

A. Efflux from tissues treated with 5 x 10^{-4}M 5-HT.
B. Efflux from tissues treated with 10^{-5}M tyramine.

o - o : untreated tissues.
x - x : tissues treated with 3 x 10^{-5}M 6-OHDA.

Significance obtained using the t-test for paired data.

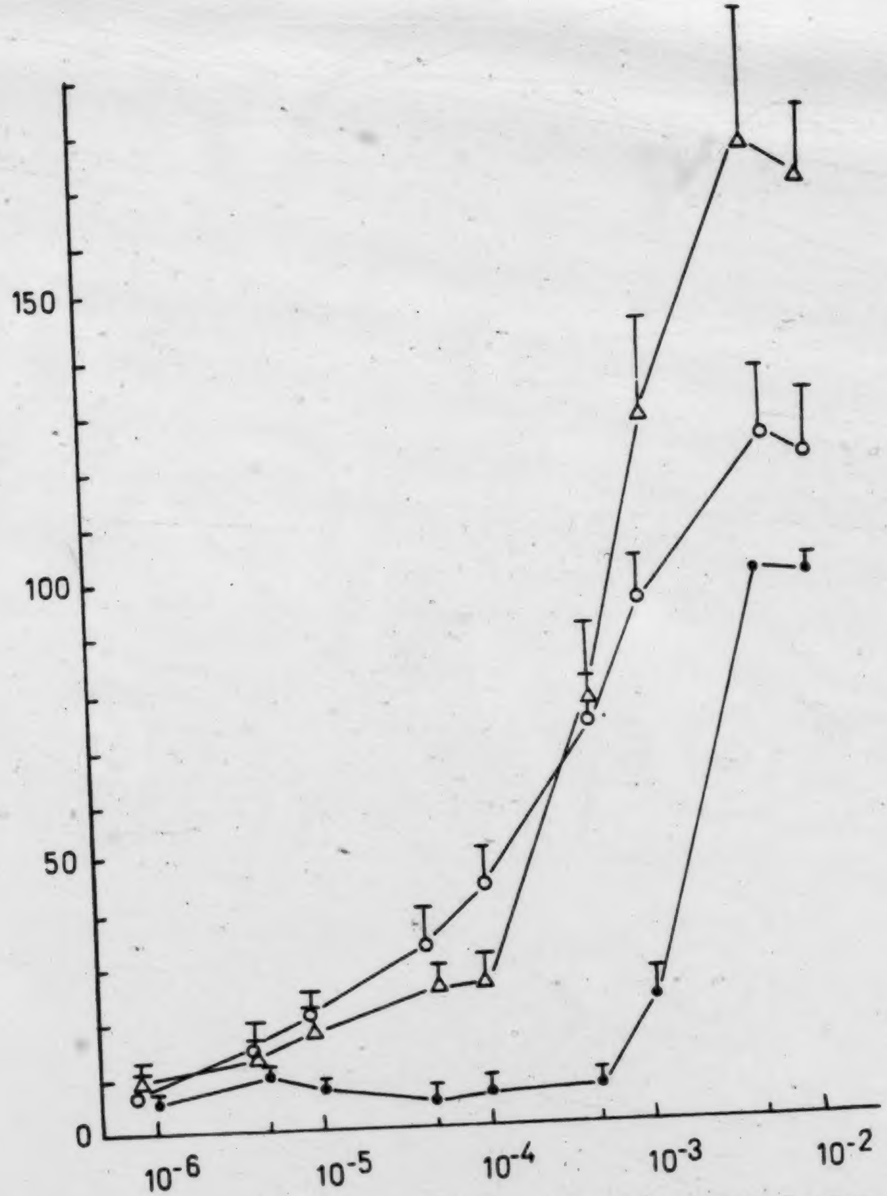


FIG. 92 Dose-response curves to histamine obtained in normal tissues and reserpinized tissues five hours after the start of the experiment, and expressed as a percentage of the initial maximum response at one hour. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. Bars represent standard errors.

● - ● : initial dose-response curve to histamine.
o - o : histamine response in reserpinized tissues after 5 hours. n - 13.
Δ - Δ : histamine response in normal tissues after 5 hours. n - 14.

component.

Further examination of other agonists however, indicated that an increase in the sensitivity of the preparation was not confined to histamine. The responses to 5-HT were found to sensitize almost to the same extent as those to histamine (Fig. 53). In addition some increase in sensitivity was also found in the case of tolazoline (Fig. 45) and betazole (Fig. 46), although the tolazoline response began to decrease at 5 hours, at a time when the responses to histamine and 5-HT were still increasing in sensitivity. These results indicate that the increase in sensitivity is thus not specific for histamine, although it does not appear to be a result of an overall increase in the ability of the preparation to contract. This conclusion is supported by the fact that there is no apparent increase in the sensitivity to acetylcholine, when preparations are first exposed to acetylcholine, then histamine and then to acetylcholine again (Fig. 93).

Another possibility is that histamine might be causing or preventing the release of prostaglandins in this preparation. Prostaglandins have been implicated in the regulation of noradrenaline release in portal vein (Greenberg, 1974, 1975) as well as in other preparations. In addition, much evidence exists to suggest that prostaglandin release may influence the sensitivity of various tissues to exogenous agonists (Jobke et al., 1976; Orehek et al., 1975). Dose-response curves obtained to histamine in the presence of indomethacin ($3 \times 10^{-6}M$), a prostaglandin synthetase inhibitor (Vane, 1971), are no different from control, however (Fig. 94). It therefore appears that prostaglandins are not involved in the sensitivity increase seen to histamine in this preparation.

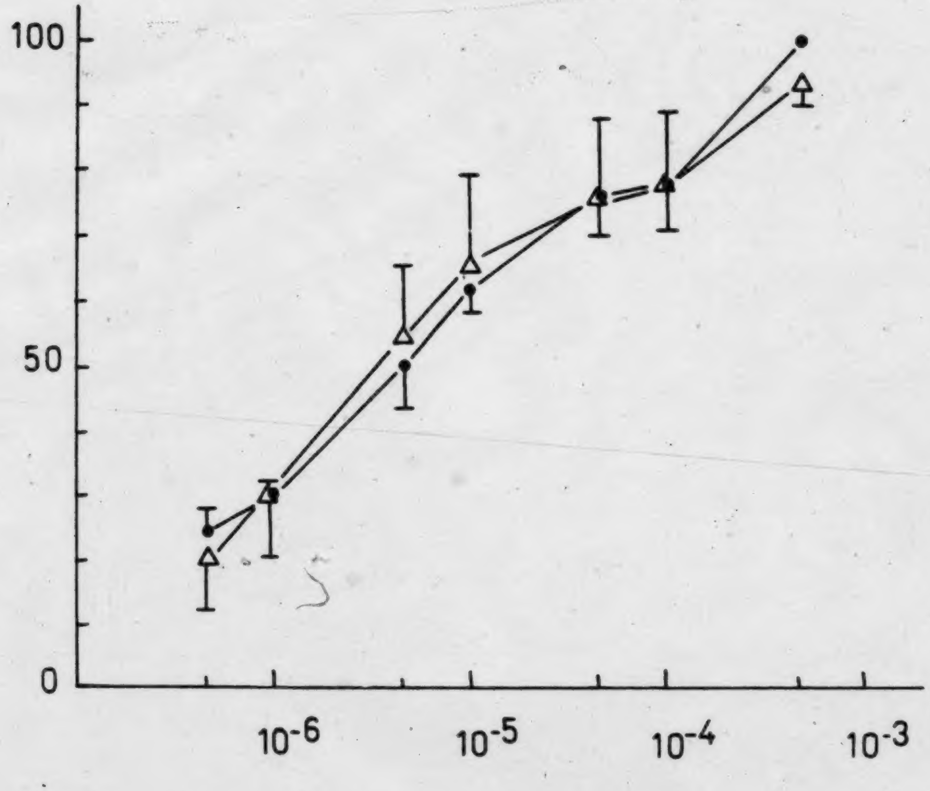


FIG. 93 Dose-response curves obtained to acetylcholine, before and after exposure of the tissues to histamine. Log molar concentration of acetylcholine as abscissa, per cent maximum response as ordinate. n = 3. Bars represent standard errors.

● - ● : initial dose-response curve to acetylcholine at one hour.

Δ - Δ : acetylcholine response at 5 hours, after exposure of tissues to histamine at 2, 3 and 4 hours.

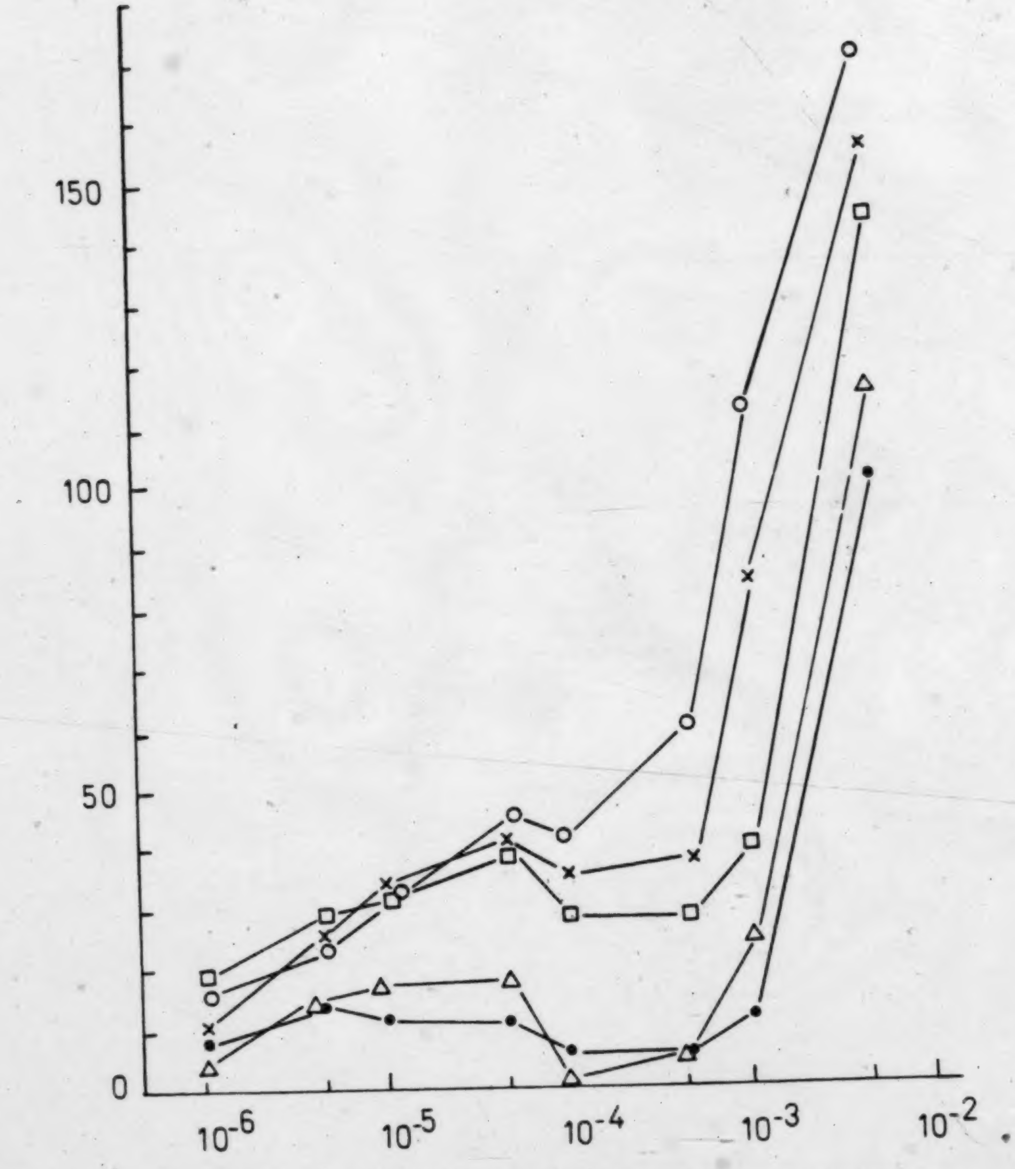


FIG. 94 The effect of indomethacin on the response to histamine. Indomethacin remained in contact with the tissues for 3 hours; the initial and the final dose-response curves were obtained in the absence of this agent. All results were expressed as a percentage of the initial maximum response at one hour. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 3.

● - ● : initial dose-response curve to histamine.
Δ - Δ : histamine response at 2 hours in presence of indomethacin.
□ - □ : histamine response at 3 hours in presence of indomethacin.
x - x : histamine response at 4 hours in presence of indomethacin.
o - o : histamine response at 5 hours in absence of indomethacin.

The fact that there was a smaller increase in sensitivity to histamine in reserpinized tissues than in control tissues led to the hypothesis that perhaps histamine, while primarily acting directly on smooth muscle, was also causing noradrenaline release and that this action became more marked with time. The release would be reduced but not abolished in reserpinized tissues because they do not appear to be fully reserpinized, and still respond to tyramine (Fig. 28). If this was so, then the increase in sensitivity to histamine should be abolished in tissues treated with 6-OHDA. This was found to be true (Fig. 95). While the maximum response in control tissues increased to 35% greater than the initial response, the maximum response in tissues treated with 6-OHDA was slightly reduced after 2 hours, and did not significantly increase on washing for another hour.

Finally, if histamine were causing release of noradrenaline by a tyramine-like mechanism, which involves active uptake and displacement of noradrenaline from sympathetic nerve terminals (Trendelenburg, 1972), then this action should be inhibited by cocaine. This was found not to be the case. When the histamine response is measured in the presence of cocaine (Fig. 96), the overall sensitization seen is not significantly different than that found in control tissues (Fig. 97). The histamine dose-response curve obtained immediately after the addition of cocaine did not sensitize over the control, but the significance of this result is unclear, since the remaining dose-response curves did show sensitization.

Time limitations prevented further exploration of this problem.

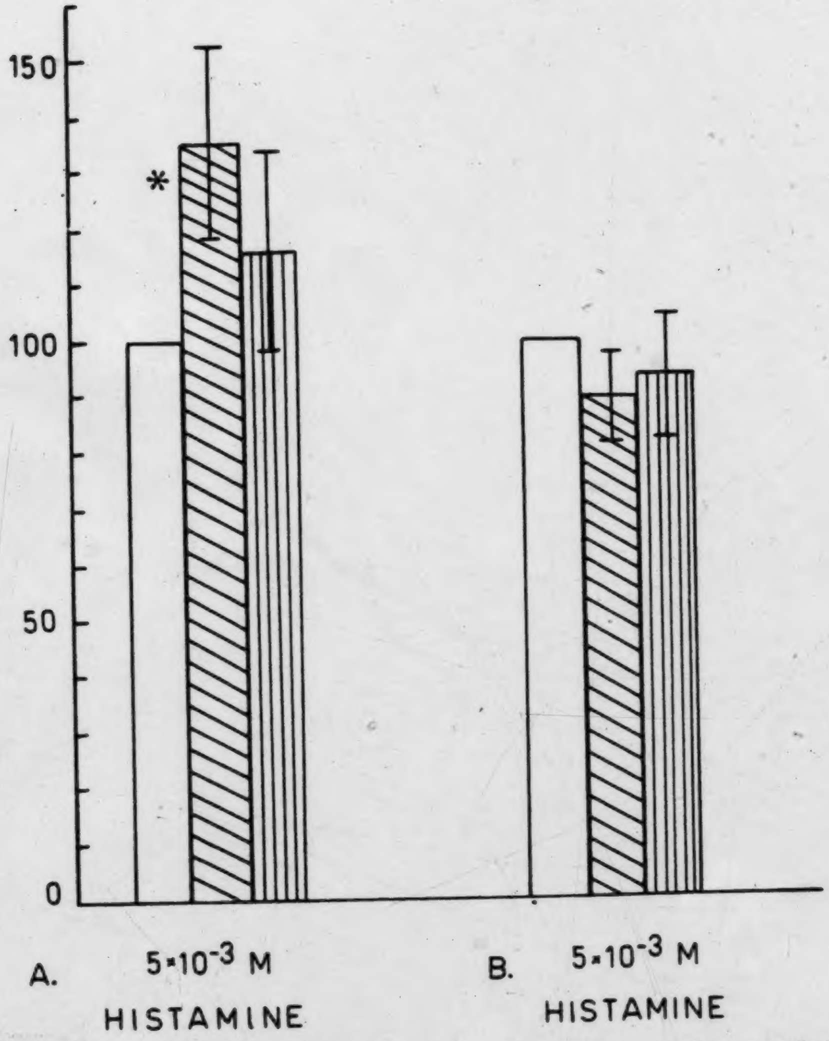


FIG. 95 Histogram illustrating the effect of 6-OHDA on the sensitization to histamine. Per cent maximum response as ordinate.

A. Control tissues.
B. 6-OHDA treated tissues.

□ : control response.

▧ : response after 250 µg/ml 6-OHDA, then 2 hour wash (6-OHDA treated tissues) response after 2 hour wash alone (control tissues).

▥ : response after further one hour wash.

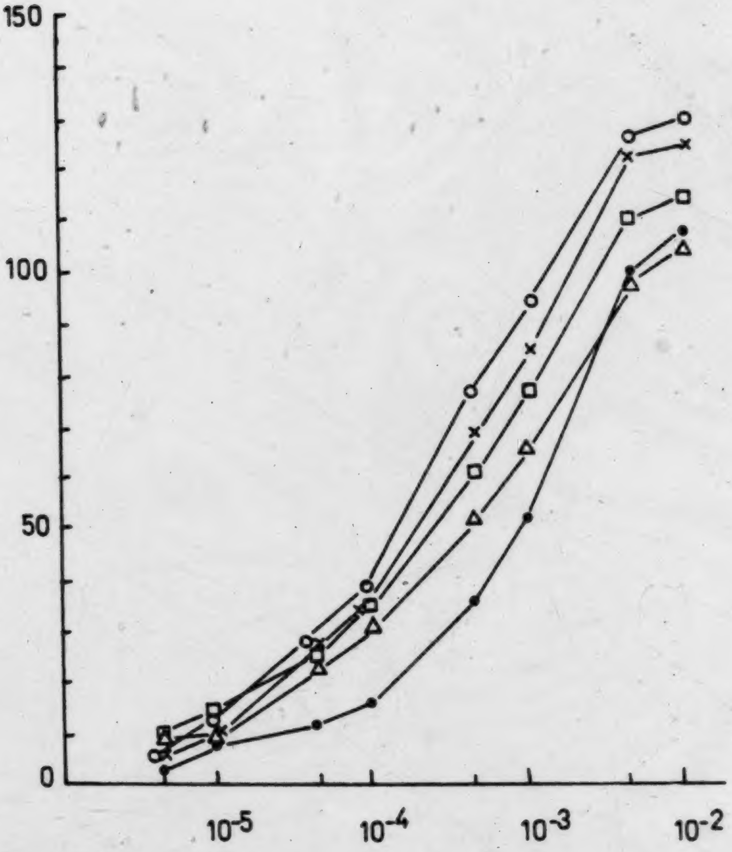


FIG. 96 The effect of cocaine on the sensitization of the histamine response. Cocaine was added immediately after the first dose-response curve was obtained, and remained in contact with the tissue for the remainder of the experiment. All results expressed as a percentage of the initial maximum response at one hour. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 6.

● – ● : initial dose-response curve to histamine.
Δ – Δ : histamine response at 2 hours.
□ – □ : histamine response at 3 hours.
x – x : histamine response at 4 hours.
o – o : histamine response at 5 hours.

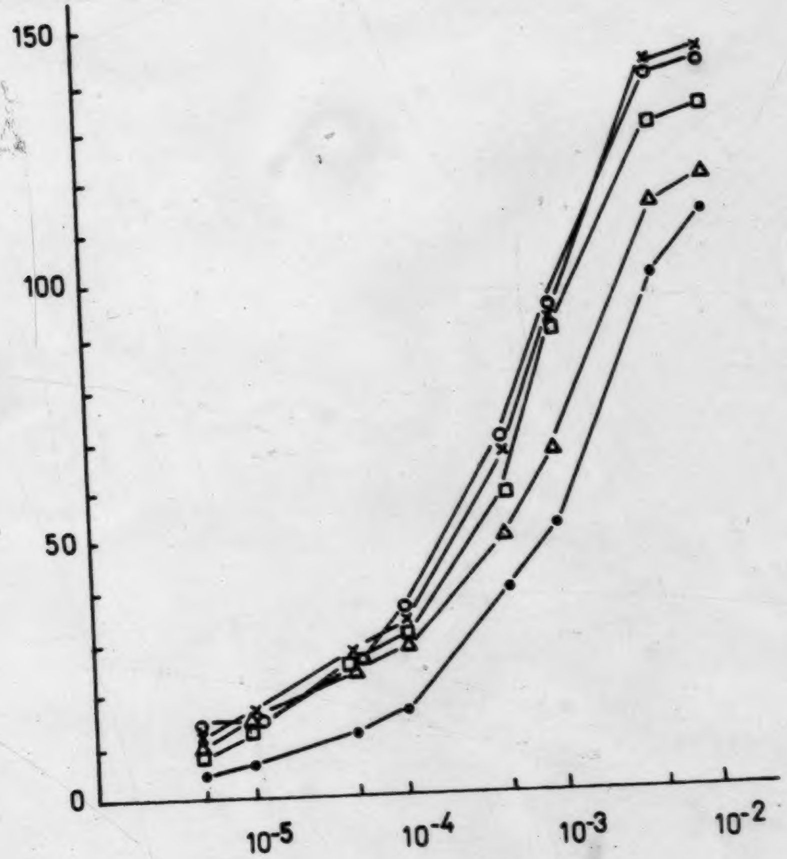


FIG. 97 Dose-response curves to histamine obtained in paired control tissues, in the absence of cocaine. All results expressed as a percentage of the initial maximum response. Log molar concentration of histamine as abscissa, per cent maximum response as ordinate. n = 6.

● – ● : initial dose-response curve to histamine
Δ – Δ : histamine response at 2 hours.
□ – □ : histamine response at 3 hours.
x – x : histamine response at 4 hours.
o – o : histamine response at 5 hours.

DISCUSSION

I SUMMARY OF RESULTS

The results described in the preceding chapter can be summarized as follows:

1. Histamine produced dose-dependent contractions of isolated spiral strips of rabbit portal vein. The response to histamine occurred over a high dose-range, and showed sensitization with time. Acetylcholine and noradrenaline also produced dose-dependent contractions in this preparation, but the responses to these agonists showed sensitization to a much smaller extent.

2. The histamine response was unaffected by cocaine, 6-hydroxydopamine (6-OHDA) treatment, or by induction of tachyphylaxis to tyramine. These procedures reduced or abolished the response to tyramine. The histamine response was not affected by atropine, in a concentration sufficient to significantly alter the response to acetylcholine. Indomethacin had no effect on the response to histamine.

3. The H_1 antagonists diphenhydramine, chlorpheniramine and antazoline all reduced the response to histamine, but only when present in high concentrations. The H_2 antagonist metiamide did not specifically block the response to this agonist. The most effective antagonists of the histamine response were the α-adrenoceptor antagonists phentolamine, azapetine and dibozane. The responses to betazole and tolazoline were also blocked by phentolamine.

4. 5-hydroxytryptamine (5-HT) produced dose-dependent contractions of rabbit portal vein, which sensitized with time. Methysergide blocked the responses to low concentrations of 5-HT, while phentolamine blocked the responses to high concentrations of this agonist. Diphenhydramine reduced the response to all concentrations of 5-HT. The

response to 5-HT was not selectively affected by 6-OHDA.

5. When desensitization was induced to 5-HT, the responses to equi-active doses of noradrenaline, histamine and 5-HT were reduced to approximately the same extent, while the acetylcholine response was unaffected. The maximal responses to histamine and 5-HT, but not to noradrenaline, were also depressed by this treatment. The response to noradrenaline, obtained in the presence of cocaine, was antagonized by high concentrations of histamine, but not by high concentrations of 5-HT.

6. Phenoxybenzamine (POB), a β-haloalkylamine, antagonized the responses to noradrenaline, histamine and 5-HT. Noradrenaline and phentolamine cross-protected the responses to all three agonists against POB blockade. Histamine, even in high concentrations, provided only partial protection of responses to itself and other agonists, while the protection provided by 5-HT had a significant non-specific component.

7. Strips of rabbit portal vein accumulated [^{3}H]-noradrenaline, the uptake of which could be reduced by cocaine. Tyramine, histamine and 5-HT all caused an increase in the efflux of [^{3}H]-noradrenaline, which was reduced in the presence of cocaine, and abolished by treatment of the tissues with 6-OHDA.

8. The responses of normal tissues to histamine, 5-HT, betazole and tolazoline were found to increase in sensitivity with time. The responses to noradrenaline and acetylcholine did not show significant sensitization. The sensitization of the histamine response was unaffected by indomethacin or atropine, but was reduced by methysergide, and in tissues taken from rabbits pre-treated with

reserpine. Histamine caused a 6-OHDA-sensitive increase in the efflux of [^{3}H]-noradrenaline; 6-OHDA treatment of isolated tissues abolished the sensitization to histamine. The histamine-induced increase in the efflux of [^{3}H]-noradrenaline was reduced in the presence of cocaine, while this agent initially blocked but did not irreversibly affect the sensitization of isolated tissues to histamine.

In the following discussion, the nature of the receptor for histamine, and the mechanism of sensitization to this agonist are considered in light of these results.

II CHARACTERIZATION OF THE HISTAMINE RECEPTOR IN RABBIT PORTAL VEIN

A. The Response to Histamine

The dose-response curve to histamine observed in isolated spiral strips of rabbit portal vein differs from that seen in most other tissues. Although the threshold response appears to occur between 10^{-7} M and 10^{-6} M histamine, the tissue does not begin to respond in a dose-dependent manner until a concentration of 10^{-4} M histamine is reached. The dose-response curve then rises sharply until the maximum occurs at approximately 5×10^{-3} M; it is possible that this is not the true maximum since the tissue does not respond to higher concentrations of histamine in a reproducible manner. The inability of the tissue to respond reproducibly to high concentrations of histamine may be due to desensitization - this phenomena has been reported to occur in other tissues in the presence of high concentrations of agonists, resulting in bell-shaped dose-response curves (Bown et al, 1973).

The histamine response in rabbit portal vein is unusual in other respects as well: the receptor for this agonist cannot be classified as either a classical H_1 receptor or the more recently defined H_2 receptor. The H_2 antagonist metiamide did not specifically block the histamine response, since the concentrations required to reduce the response to this agonist also blocked the responses to other agonists. On the other hand, the H_1 antagonists diphenhydramine and chlorpheniramine appeared to produce a specific blockade of the response to histamine, but only in the presence of high concentrations of these antagonists. Much lower concentrations of the α-adrenergic antagonist phentolamine were effective at blocking the response to histamine, and these same concentrations of phentolamine also blocked the response to noradrenaline. Phentolamine has been reported to antagonize the histamine response in guinea pig ileum, which contains an H_1 receptor system, and guinea pig ventricle, which contains an H_2 receptor system, but in both cases, higher concentrations of phentolamine were necessary than those required to block the histamine response in portal vein (MacLeod et al, 1978). The histamine response in strips of rabbit vena cava (containing an H_1 receptor system) was also reported to be blocked by phentolamine, but the pA_2 value of phentolamine against histamine was much greater than that against noradrenaline (Gulati et al, 1968). Thus the inability of metiamide to antagonise the histamine response, together with the relatively weak actions of the H_1 antagonists, and the sensitivity of the system to phentolamine, imply that the receptor cannot convincingly be placed in either the H_1 or the H_2 class.

Of the numerous possible explanations for these results, one of the most likely is that histamine is acting indirectly through the release of noradrenaline in this tissue. This could be mediated by an H_1 type of receptor which is poorly accessible to classical H_1 antagonists, and the actions of phentolamine are thus explained by it's action at the α-adrenergic receptor. This possibility is discussed in the next section.

B. The Mechanism of Action of Histamine

Noradrenaline is stored in vesicles located in sympathetic nerve terminals, from which it can be released in response to various stimuli (von Euler, 1972). The means by which sympathomimetic agents can cause release of noradrenaline is still incompletely understood, but at least two mechanisms are recognized. The first of these occurs via a 'tyramine-like' action, which is cocaine-sensitive. Tyramine has been shown to be taken up into adrenergic nerve terminals by active transport through the neuronal membrane, after which it causes stoichiometric displacement of noradrenaline from storage vesicles (Trendelenburg, 1972). Most sympathomimetic amines have been reported to act in this manner, and this has also been proposed as the mechanism of action of 5-HT in cat splenic strips (Innes, 1962 b). The second mechanism is a 'DMPP-like' action; DMPP and other nicotinic drugs have been reported to cause noradrenaline release through depolarization of the nerve terminal with resultant exocytosis of noradrenaline-containing vesicles (Musholl, 1970). This action can be inhibited by hexamethonium, colchicine and decreased calcium. 5-HT has been reported to release noradrenaline by a DMPP-like mechanism in heart (Fozard and Mwaluko, 1976). Histamine has only rarely been reported to cause nordrenaline release, and the mechanism has been examined in only one case. The authors reported that release of noradrenaline by histamine could by inhibited by hexamethonium, implying that the second mechanism is correct (Everett and Mann, 1967). However, the effects of cocaine were not examined in this preparation.

Treatment of tissues with drugs which deplete the neuronal stores of noradrenaline results in abolition of responses to all indirectly acting agents. Reserpine is often used as the depleting agent, although it acts slowly and animals must be pre-treated before sacrifice. It has been shown to cause almost complete depletion of tissues such as the heart and spleen (Iversen, 1967). Reserpine is believed to yield the most reliable quantitative results, when the action of agents which may have a partially direct and partially indirect action are being considered (Trendelenburg, 1972), and thus would be the ideal tool to examine the mechanism of action of histamine in portal vein. However, pre-treatment of rabbits with up to twice the normal dose of reserpine failed to abolish the response to tyramine in this preparation. Although tyramine has been reported to have some direct actions at very high concentrations (Pluchino, 1972), it is unlikely that the responses to low concentration of this agent in portal vein are due to a direct action on smooth muscle and it must be concluded that the noradrenaline stores in sympathetic nerve terminals have not been completely depleted. It is possible that the portal vein is particularly resistant to reserpine or that although some depletion has taken place, sufficient noradrenaline remains to provide a substantial response to tyramine. This second possibility has been shown to be true in other tissues, although it has usually been found that acute pre-treatment with large doses of reserpine abolishes the response to tyramine (Antonaccio and Smith, 1971).

Portal veins were subjected to three other procedures designed to reduce the effects of indirectly acting agents (Results I-D). The first of these, induction of tachyphylaxis to tyramine, resulted in a significant reduction in the response to tyramine, and had no significant effect on the response to histamine. These results indicate that tyramine and histamine are not causing release of noradrenaline from the same stores. Only partial depletion of the total noradrenaline stores occurs even on induction of tachyphylaxis to a very high dose of tyramine, however (Weiner et al, 1962), so it cannot be assumed on the basis of this experiment alone that histamine is acting directly on smooth muscle. The persistence of the response to histamine in the presence of cocaine however, in a concentration which significantly reduced the response to tyramine, provides furthur evidence that histamine is not causing release by a mechanism similar to that of tyramine. The concentration of cocaine employed did not block the response to tyramine completely, although Hughes (1972) found that this concentration provided optimal blockade of noradrenaline uptake. There is some evidence that noradrenaline and cocaine can compete for uptake (Iversen, 1967) so it seems likely that tyramine in sufficient concentration can also overcome the inhibition of uptake produced by cocaine. The reduction in the response to tyramine in the presence of cocaine suggests that if histamine were acting through uptake via a cocaine-sensitive site as well, the response to this agonist would also be reduced under these circumstances. Since the response to histamine is not only unaffected by induction of tachyphylaxis to tyramine, but is also resistant to blockade by cocaine, any release of noradrenaline by histamine must be occurring from tyramine-resistant stores, by a mechanism insensitive to cocaine.

If histamine were acting by some such mechanism, the response to this agonist would be abolished by chemical denervation with 6-OHDA. This neurotoxic compound is highly selective for sympathetic nerves. It is taken up into adrenergic nerve terminals by the active transport mechanism of the neuronal membrane, where it then causes degeneration of the nerve terminals (Malmfors and Sachs, 1968: Jonsson, 1976). It is not 6-OHDA but products of its intraneuronal auto-oxidation which are thought to be cytotoxic; these products are believed to bind to and cross-link intracellular proteins, causing their inactivation (Jonsson, 1976; Rotman and Creveling, 1976). The method chosen for treatment of tissues with 6-OHDA in the present experiments was a modification of that used by Aprigliano et al (1976), in which tissues are exposed in the organ bath to 6-OHDA, washed extensively and then exposed to drugs. The authors reported that this procedure resulted in degenerative changes in sympathetic nerve terminals of rat portal vein, when tissues were examined electron microscopically. In addition, no catecholamines were detectable with fluorescent histochemical techniques.

In the first set of experiments involving 6-OHDA, the response to tyramine was completely abolished. The histamine and acetylcholine responses were reduced to the same extent, leading to the suggestion that 6-OHDA might be having a non-selective effect on the smooth muscle.

Although 6-OHDA has high affinity for the neuronal uptake mechanism, it is possible that some 6-OHDA is also taken up extraneuronally into smooth muscle cells, causing loss of contractile function in these cells, manifested by a small reduction in the responses to histamine and acetylcholine. In order to determine whether 5-HT was acting directly or indirectly, these experiments were repeated, and in the second set of experiments, a small response to tyramine (10°/₀ of control) could still be detected. The responses to all the other agonists tested, (5-HT, histamine and acetylcholine) were again slightly reduced and to the same extent, so it was decided not to increase the exposure time or the concentration of 6-OHDA. However, it has been reported that tyramine does not cause release equally from all stores of noradrenaline, and in fact at least two stores of noradrenaline can be differentiated, based on their susceptibility to tyramine (Potter et al., 1962). Just as the response to tyramine can be abolished by induction of tachyphylaxis, without complete depletion of neuronal noradrenaline, pre-treatment of an animal with reserpine can result in almost complete abolition of the response to tyramine without significantly affecting the response to electrical stimulation (Antonaccio and Smith, 1971). Some studies on the release of [^{3}H]-noradrenaline by tyramine have indicated that tyramine can cause preferential release of noradrenaline (Iversen, 1967).

The possibility thus existed that some noradrenaline stores might still remain in the tissue even after 6-OHDA treatment, and that these were resistant to tyramine, resulting in only a small response to this agent, but subject to greater release by histamine or 5-HT. In order to examine this possibility, tissues were stimulated electrically before and after treatment with 6-OHDA. It was felt that noradrenaline stores resistant to tyramine would be released on electrical stimulation, since other studies have suggested that this is the case (eg. Antonaccio and Smith, 1974; Trendelenburg et al, 1962). In fact it was found that there was no significant difference between the response to tyramine and that to electrical stimulation after treatment of tissues with 6-OHDA. When the responses to these two stimuli were reduced to less than 15% of the control value, there was no specific reduction in the responses to histamine and 5-HT (Results I-G).

These results, then, strongly suggest that both histamine and 5-HT have their main action by some mechanism other than an indirect sympathomimetic effect. Experiments in which the efflux of $[^3H]$-noradrenaline was monitored provide furthur support for this action of histamine and 5-HT.

Prior to monitoring the effects of drugs on the efflux of $[^3H]$-noradrenaline, the uptake of this substance into portal vein was measured, in the presence and absence of cocaine (Results, II). A cocaine-sensitive component of $[^3H]$-noradrenaline uptake was present, indicating that neuronal uptake was occurring, and there was also a significant cocaine-insensitive component, indicating that extraneuronal uptake of this substance also occurs in portal vein. This is in agreement with results presented by Hughes (1972) who showed that noradrenaline action could be terminated by both neuronal and extraneuronal uptake in portal vein. He also found that although neuronal uptake is generally more important for removal of noradrenaline, in the presence of cocaine, the relative importance of extraneuronal uptake increased significantly. Thus in the experiments reported here, simply subtracting the cocaine-sensitive uptake from the cocaine-insensitive uptake would be expected to give a erroneously high estimate of extraneuronal uptake.

Bevan et al (1974) have shown that an inverse relationship exists between the uptake of [^{3}H]-noradrenaline and the ED_{50} for noradrenaline in a series of rabbit veins; the higher the ED_{50} for noradrenaline, the lower the neuronal uptake of noradrenaline. The results presented here agree with their findings; the ED_{50} for noradrenaline in rabbit portal vein is approximately $2x10^{-7}$ M, while the T/M ratio is about 4.5 gm/ml after one hour. The density of adrenergic innervation of a tissue can be directly related to the amount of uptake of [^{3}H]-noradrenaline, and this is also in agreement with our results: the portal vein is some what sparsely innervated, compared with tissues such as the vas deferens. However, the T/M ratio for uptake in rat portal vein after one hour has been reported to reach approximately 13 ml cleared/gm tissue (Ljung et al, 1973), which is almost three times as great as that found in the present study. Ljung et al (1973) have suggested that differences in the T/M ratio between tissues can be related to differences in the noradrenaline content; the noradrenaline content of rat portal vein (Haggendal et al, 1970) has been reported to be higher than that of rabbit portal vein (Huges, 1972). In addition, the difference might be attributable to differences in experimental technique, particularly with regard to obtaining the tissue weights. Due to the very small size of most of the segments of portal vein used in these experiments, the weights measured were found to be extremely sensitive to alterations in moisture content. In these experiments tissues were weighed immediately after blotting, and this weight was used to determine the T/M ratio. If the tissues were allowed to stand for any length of time the tissue weights decreased by as much as 50%, which would result in a doubling of the T/M ratio.

Tissues were loaded with 3×10^{-7}M [^{3}H]-noradrenaline, then efflux of tracer was measured under a variety of conditions (Results II). This concentration of noradrenaline was reported by Hughes (1973) to mix homogeneously with endogenous noradrenaline stores in rabbit vas deferens and portal vein, and therefore any release of labelled noradrenaline obtained in the presence of drug would be expected to reflect the release caused by this drug under normal circumstances.

Histamine and 5-HT, as well as tyramine, were found to cause release of [^{3}H]-noradrenaline, in both the presence and absence of inhibitors of monoamine oxidase and catechol-o-methyl transferase. The increased release produced by histamine and 5-HT was not significantly different from that produced by tyramine. However, the release caused by all three amines was significantly reduced by cocaine in the same concentration as that which significantly inhibited the pharmacological response to tyramine but did not affect the response to histamine or 5-HT. Treatment of tissues with 6-OHDA reduced the efflux coefficients of noradrenaline compared to the control and abolished the increase in efflux to all three agonists. This procedure abolished only the pharmacological response to tyramine and did not appear to specifically affect the responses to 5-HT or histamine.

It seems highly unlikely that a store of noradrenaline exists in rabbit portal vein, from which histamine and 5-HT can cause release, which is insensitive to 6-OHDA, cocaine and induction of tachyphylaxis to tyramine, and which cannot be detected either pharmacologically or biochemically. Rather, it must be assumed that histamine and 5-HT are not acting by causing release of noradrenaline, although the possibility that a component of their action is indirect is discussed in a later section. The much less likely possibility that some other endogenous transmitter is being released by histamine has been partially answered by studies reported here involving atropine and indomethacin. Neither antagonist has any significant action as a histamine antagonist in this preparation, suggesting that neither acetylcholine nor prostaglandins are involved in the response to this agonist. Thus the most likely explanation of the results discussed above is that histamine acts directly on the smooth muscle of rabbit portal vein.

C. The Histamine Receptor

There are at least three possible receptors through which histamine could be acting in rabbit portal vein. There are: (1) a histamine receptor, unusually resistant to histamine antagonists; (2) a receptor for 5-hydroxytryptamine; or (3) a non-specific amine receptor with a close resemblance to the α-adrenoceptor.

The evidence against the possibility that the receptor for histamine in rabbit portal vein is a typical H_1 or H_2 receptor has been discussed (This chapter, I-A). It also appears unlikely that this preparation contains a histamine receptor unusually resistant to blockade by diphenhydramine or chlorpheniramine, or that access of these antagonists to the receptor is limited. Phentolamine blocked the response to histamine at a much lower concentration (10^{-8} M) than that necessary for inhibition by the H_1 antagonists (10^{-6} M), suggesting that access to the histamine receptor is not restricted. Antazoline is classified as an H_1 antagonist (Douglas, 1975) but structurally it is very similar to phentolamine; high concentrations of this antagonist were necessary to block the histamine response, and in this case the response to noradrenaline was also blocked by the same concentrations of antazoline. Similar doses of this antagonist were able to block the noradrenaline response in strips of vena cava from the rabbit, which appear to contain typical α-adrenergic receptors (Gulati et al, 1968). The receptor for histamine in rabbit portal vein thus is unusually resistant to blockade by a number of H_1 antagonists. Other evidence suggests that rather than specifically blocking the histamine response, the H_1 antagonists are simply restricting the response to agonists in general in a non-specific manner. Of the three agonists most extensively studied in this preparation, histamine was shown to be the least potent, while 5-HT is intermediate and noradrenaline is the most potent. Thus would imply that the histamine response could be the most susceptible to restriction, resulting in what initially appears to be specific blockade. This is supported by the results of two other experiments. First diphenhydramine depresses the response to 5-HT over the entire dose-response curve, although other evidence indicates that 5-HT may be acting on two sets of receptors (Results, I-G). Secondly, the histamine response is not protected against phenoxybenzamine(POB) blockade by diphenhydramine present in a concentration ($5x10^{-6}$ M) sufficient to reduce the maximum response to histamine to less than 40% of control (Results I-I).

The action of agents which act as agonists at histamine receptors in other preparations also suggest that the receptor for histamine in portal vein is unusual. Betazole has been found to be more active at H_2 than H_1 receptors, as has 4-methylhistamine, while n-methyl-histamine is more active at H_1 receptors. Ethyl-2-pyretamine has been found to be a partial agonist at H_1 receptors. Betazole and n-methyl-histamine were both found to be as potent as histamine in the rabbit portal vein, while 4-methyl-histamine and ethyl-2-pyretamine were almost inactive. Thus, in this preparation, one H_1 agonist and one H_2 agonist were close to equi-potent, while, the other H_1 and H_2 agonists were almost inactive. These results, together with the results obtained with the H_1 and H_2 antagonists, suggest that histamine is not acting at a typical histamine receptor in rabbit portal vein. The blockade of the histamine and betazole responses with low concentrations of phentolamine suggest that rather than acting at a histamine receptor at all, these agonists may be acting at a receptor for another agonist, either 5-HT or noradrenaline.

Phentolamine has been reported to block the 5-HT receptor in rabbit aortic strip; it has one-tenth the affinity for the 5-HT receptor as for the adrenergic receptor (Furchgott, 1972). 5-HT has been reported to act at the adrenergic receptor in rabbit ear artery, and this interaction could be blocked with phentolamine (Apperley et al, 1976). Since the response to 5-HT in rabbit portal vein was blocked with phentolamine, the two remaining possibilities concerning the receptor for histamine had to be considered. The first was that histamine and 5-HT are acting at a common receptor, distinct from that for noradrenaline, and the second that all three agonists are acting at a common receptor.

On the basis of the evidence described below, the second possibility seems more likely. The histamine response was blocked with two other α-adrenergic blocking agents, azapetine and dibozane, in concentrations similar to those necessary to block the response to noradrenaline. However, whether these antagonists also block the response to 5-HT in portal vein and in other tissues is unknown. The responses to low concentrations of 5-HT were blocked by methysergide, (which is a typical antagonist at 5-HT 'D' receptors in other tissues, (see Fozard, 1975)), but not by phentolamine. The opposite situation occurred at high concentrations of 5-HT: these responses were blocked by phentolamine but unaffected by methysergide. Since the concentration of phentolamine necessary to block the 5-HT response was similar to that necessary to block the noradrenaline response to the same extent, and 5-HT is probably acting directly on smooth muscle, it appears that 5-HT could be acting at the same receptor as noradrenaline.

The results of the experiments involving cross-desensitization of the responses to noradrenaline, 5-HT and histamine provide support for this hypothesis (Results I-H). The mechanism by which desensitization occurs is not well understood, but studies in a number of preparations have indicated that specific desensitization occurs at the level of the receptor, and may involve a conformational change in the receptor from an active to a less active form (Rang and Ritter, 1970; Kenakin and Cook, 1978). Specific desensitization affects only those agonists acting at a particular receptor, the responses to agonists at other receptors being unaffected by this treatment. In the initial experiments, maximum concentrations of each agonist were employed to determine the effects of desensitization on the responses to that agonist. In these experiments, the responses to histamine and 5-HT were reduced to 50% of the control value, while the noradrenaline response was unaffected. It has been reported that no cross-desensitization occurs between α-adrenoceptor agonists of the imidazoline class and those of the phenylethylamine class, and this has been used as evidence to suggest the existence of two different sites on the α-adrenoceptor at which agonists can act (Ruffolo et al, 1977). However it is unlikely that this situation occurs in portal vein. A comparison of the dose-response curves to noradrenaline, histamine and 5-HT obtained on the same preparations showed clearly that if all three agonists are acting on the same receptor, then noradrenaline is acting as a full agonist and histamine and 5-HT are acting as partial agonists, even after maximal sensitization has occurred.

Rang and Ritter (1970) reported that whereas dose-response curves to full agonists were shifted by desensitization, while a maximum response similar to the control response could be obtained, dose-response curves to partial agonists were both shifted and depressed by the same procedure. Therefore using doses close to the ED_{50} would be expected to provide a more accurate measure of the relative effects of desensitization on the response to noradrenaline, histamine and 5-HT. This in fact turned out to be the case; using equi-active doses of all three agonists in the ED_{50} range, it was found that the responses to all of them were reduced by approximately the same amount after induction of desensitization. The response to acetylcholine, when used in in a concentration in the same range as the other agonists, was unaffected by this procedure, indicating that specific desensitization has taken place.

If all three agonists are acting at the same receptor, a number of other predictions concerning the actions of these agonists can be made. Partial agonists can act as partial antagonists of the response to full agonists, when present in sufficient concentration, by limiting the access of full agonists to the receptor (Ariens et al, 1964). In order to demonstrate this effect however, there should be no other means by which the partial agonists could potentiate or decrease the response to the full agonists, thus either enhancing or diminishing the apparent degree of blockade produced.

Initial attempts to block the noradrenaline response in portal vein with high concentrations of histamine or 5-HT were unsuccessful. However, 5-HT has been shown to be accumulated into adrenergic neurons in vas deferens, (Thoa et al, 1969), and also to inhibit the accumulation of noradrenaline into chromaffin granules in the adrenal medulla (Kirshner, 1962; Carlsson et al, 1963). Although no such action has been reported for histamine, it seemed reasonable that the high concentrations of these agonists necessary to interfere with the noradrenaline response could result in an interference with the neuronal uptake of noradrenaline, thus potentiating the noradrenaline response and obscuring any receptor blockade. For this reason dose-response curves to noradrenaline were obtained in the presence of cocaine, and then histamine or 5-HT was added, with cocaine still present in the bath. Then a furthur dose-response curve was obtained to noradrenaline. By blocking neuronal uptake with cocaine, it was hoped that any furthur sensitization of the noradrenaline response would be minimized. The results indicated that under these conditions histamine, in a concentration of $5x10^{-3}$ M, was able to antagonize the response to noradrenaline, while the concentration of 5-HT which produced the maximum response to this agonist ($5x10^{-4}$ M) did not significantly affect the noradrenaline response (Results I-H). The antagonism produced by histamine of the response to noradreanline is difficult to describe, but could result from histamine having more than one action even in the presence of cocaine. The ability of both histamine and 5-HT to block the accumulation of noradrenaline into smooth muscle in rabbit ear artery has been reported (Buchan et al, 1974). The inhibitory effect of histamine on the extraneuronal uptake of noradrenaline occurred at concentrations ranging from $3x10^{-4}$ M to $3x10^{-3}$ M; these concentrations of histamine are similar to those used in the present experiments. As already mentioned, Hughes (1972) has shown that when neuronal uptake in rabbit portal vein is inhibited, extraneuronal uptake for the removal of noradrenaline becomes relatively more important. By inhibiting neuronal uptake with cocaine in the present study, possibly the balance shifted towards extraneuronal uptake. When 5-HT or histamine were added, they might have then caused a blockade of the extraneuronal uptake, potentiated the noradrenaline response, and again obscured any receptor antagonism produced. This hypothesis could have been tested by the addition of an antagonist of extraneuronal uptake, used in conjuction with cocaine. Rather than pursue this line of questioning however, and risk having additional non-specific effects due to the use of more drugs, it was decided to switch to other means of receptor differentiation.

The results of the receptor self-protection and cross-protection experiments are summarized in Table II. Noradrenaline protects the responses to noradrenaline, histamine and 5-HT, and the protection afforded by noradrenaline seems to be specific, since the acetylcholine response is not protected by this agonist. Phentolamine is also quite effective at protecting the responses to noradrenaline, histamine and 5-HT, even in the relatively low concentration used here. The protection provided by histamine and 5-HT does not appear to be so well-defined. Histamine, even when present in very high concentrations, did not provide much protection of the responses to any of the other agonists. This may simply reflect it's position as the agonist with the least apparent affinity for the receptor at which it acts. 5-HT, on the other hand, protected too well, appearing to protect the acetycholine response as well as the responses to histamine and noradrenaline. It appears that 5-HT in high concentrations can bind to a wide range of different sites in smooth muscle, since this agonist does not seem to have any direct action through cholinergic receptors.

The problems which arise from the use of receptor protection experiments have already been discussed (Results, I-I). Waud (1962) suggested that high doses of protecting agents could bind non-specifically to other sites through which the protecting agent has no action, and this was confirmed by the experiments of Moran et al (1967), who found that noradrenaline protected many non-specific

TABLE II

Summary of results of protection experiments

Protecting Agent	Response to be protected			
	Noradrenaline	Histamine	5-HT	Acetylcholine
Noradrenaline	+++	+++	+++	0
Phentolamine	+	+	+	NA
Histamine	+	+	0	0
5-HT	+	+++	+++	+++
Diphenhydramine	NA	0	NA	NA

+++ = Full protection
+ = Partial protection
0 = No protection
NA = Experiment not performed.

sites as well as the α-adrenergic receptor. This evidence, together with the results of protection experiments with 5-HT and histamine described above, suggest that the protection provided by these two agonists is probably non-specific, at least in part. However, both noradrenaline and phentolamine were used in lower concentration in these experiments. Although it is difficult to exclude a non-specific component of the protection by these two agents, in the case of noradrenaline this protection did not extend to the acetylcholine receptor, and under these circumstances, both the response to histamine and that to 5-HT were significantly protected.

The results discussed throughout this section can be summarized as follows:

(1) The responses to noradrenaline, 5-HT and histamine are all blocked by the same concentrations of phentolamine. Azapetine and dibozane also antagonize the responses to both histamine and noradrenaline.

(2) Within any one preparation, the maximum response to histamine reaches 75% and the maximum response to 5-HT reaches 85% of the maximum response to noradrenaline (100%).

(3) Desensitization of the response to 5-HT results in cross-desensitization to equiactive doses of histamine, 5-HT and noradrenaline. The same procedure results in cross-desensitization to maximum concentrations of histamine and 5-HT, while the maximum noradrenaline response remains unaffected.

(4) It was not possible to block the noradrenaline response in untreated tissues with high concentrations of 5-HT or histamine. In the presence of cocaine, histamine (5×10^{-3} M) blocked the noradrenaline response but the antagonism produced had some unusual

features.

(5) Noradrenaline and phentolamine protected the responses to noradrenaline, histamine and 5-HT against POB blockade. The protection provided by these agents appeared to be specific. The protection provided by histamine and 5-HT was less well-defined than that produced by noradrenaline and phentolamine, and due to the high concentrations employed, also appeared to have a non-specific component.

These results, together with the evidence supporting the direct action of histamine and 5-HT, strongly suggest that these two agonists and noradrenaline are acting at a common receptor, which appears to be a non-specific amine receptor. This receptor bears closest resemblance to the α-adrenergic receptor, in that noradrenaline acts as a full agonist, and phentolamine and the other α-adrenergic antagonists appear to block the response to all agonists acting through it.

It is possible that this receptor represents another sub-type of α-adrenoceptor. Recent evidence indicates that the population of α-adrenergic receptors may not be as homogenous as previously beleived. For instance, Barker et al, (1977) compared the isomeric activity ratios (the ratio of the concentrations of (+)-and (-)- isomers of a drug necessary to produce the same response) of (+)-and(-)-noradrenaline in a number of tissues. This ratio is believed to differ between tissues only if there are differences in the characteristics of the receptor, once stereospecific sites of drug loss have been eliminated. These authors found significant differences between the isomeric activity ratios of (+)- and (-)-noradrenaline when these were measured in a large number of tissues. They suggested that these tissues could be divided into three groups based on the ratio obtained for each tissue. This, they suggested, reflects differences in the α-adrenoceptor in these tissues, even though no significant difference in pA_2 values of α-adrenergic antagonists measured against noradrenaline was observed. This conclusion is supported by evidence obtained with an apparently selective antagonist of α-adrenergic receptors. Prazosin is thought to act primarily by inhibition of α-adrenergic receptors, yet while it blocks all pressor responses to phenylephrine, it was found to block only the responses to higher doses of noradrenaline in the pithed rat preparation (Bentley et al, 1977). Phentolamine was found to inhibit responses to all concentrations of both agonists in this preparation. Bentley et al (1977) suggest that there are at least two types of post-synaptic α-receptor, one sensitive to prazosin and one insensitive to this compound, and both sensitive to blockade by phentolamine. Phenylephrine acts only through the prazosin-sensitive receptor, while noradrenaline acts through both, activating the prazosin-insensitive receptor when noradrenaline is present in high concentrations.

Cerebral arteries also appear to contain α-receptors which are different from those in the peripheral circulation, on the basis of the relative potency ratios of a number of agonists (Edvinsson and Owman (1974). Duckles and Bevan (1976) have presented evidence suggesting that rabbit basilar artery contains two distinct sets of adrenoceptors: one of low sensitivity to noradrenaline and phentolamine and one of high sensitivity to these agents. They suggest that these two receptor types may be present in a number of tissues, but the low sensitivity receptors are normally hidden by responses to low concentrations of agonists of the sites of high sensitivity. It is possible that in rabbit portal vein, histamine and also 5-HT could be acting at such a site of low sensitivity, which Duckles and Bevan (1976) also suggest is less discriminatory than the highly sensitive receptor. The action of histamine and 5-HT would be revealed because of the lack of highly sensitive receptors specific for these agonists. Noradrenaline, normally present in relatively low concentrations, could be acting at the site of higher sensitivity, but when used for protection in a concentration of 10^{-4}M, it could interact with the less sensitive receptor to give protection of the responses to 5-HT and histamine. Although this explanation is plausible, the results of the cross-desensitization experiments suggest that even in relatively low concentrations (2×10^{-7}M), noradrenaline is acting at the same receptor as histamine and 5-HT.

While the majority of evidence supports the view of a common receptor, some findings could, by themselves, imply some other mechanism. It would be surprising, if a common receptor does exist, for some agonists to be blocked competitively, while others were blocked non-competitively, by the same antagonists. Initially it appears that phentolamine is blocking the noradrenaline and 5-HT responses competitively, but is a non-competitive antagonist of the histamine response. However, when attempts were made to analyse the nature of the blockade with double reciprocal plots, no clear conclusions could be drawn, and it is concluded that the nature of the antagonism is unknown. It is possible (and perhaps probable) that the apparent differences in blockade arise from the insensitivity of the tissue to histamine, and cannot be regarded, on the basis of evidence presented here, to be a major objection to the hypothesis of a common amine receptor.

Thus it appears at the present time that histamine, 5-HT and noradrenaline are interacting with an unusual variety of α-adrenoceptor, which responds to other amines apart from noradrenaline. Whether betazole and tolazoline are also acting at this receptor is not clear; although the blockade of the response to these agonists with phentolamine suggests that this is a possibility, not enough information is available to specify the site of action of these two agents.

A non-specific receptor of the type discussed here has not been described in other tissues, although it is possible that further examination of vascular smooth muscle from the rabbit and other species will reveal that this type of receptor exists elsewhere. However, preliminary studies in this laboratory of the histamine response in portal vein taken from the monkey, indicated that histamine is acting at a typical H_1 receptor. Gulati et al. (1968) also reported that histamine was acting at a typical H_1 receptor in rabbit posterior vena cava.

III THE MECHANISM OF SENSITIZATION TO HISTAMINE

There are many possible mechanisms by which the response of a tissue to a drug may increase during an experiment. The most obvious involve increased access or decreased removal of the drug from the receptor, and these processes were recently analyzed by Kalsner (1976). In the sympathetic system, sensitization to noradrenaline, when specific, usually involves interference with the uptake of noradrenaline, and this is usually achieved by means of drugs or denervation. However, the sensitization seen to histamine and 5-HT in rabbit portal vein occurs in untreated tissues, in which no effort has been made to alter the rate of breakdown or uptake of these agonists. The sensitization is not non-specific: the responses to acetylcholine and noradrenaline show no such significant sensitization and in addition, treatment of a tissue with histamine does not result in an increase in sensitivity to acetylcholine. These results suggest that the sensitization seen to histamine is mediated by an effect at the receptor.

There are at least three major routes by which this could occur:

(1) An increase in the total number of receptors for histamine;

(2) An increase in the concentration of histamine at the receptor, by either increased access or decreased removal, or an increase in the ability of the receptors present to combine with histamine; or

(3) Histamine could be releasing an agent which causes either a real or an apparent increase in sensitivity to histamine.

The last possibility appears to be the most appealing, on the basis of the apparent inability of tissues taken from reserpinized rabbits to sensitize to the same extent as normal tissues. As mentioned earlier, although tissues were not reserpinized totally, it is possible that they were partially affected by this drug. This suggestion could be confirmed by direct assay of noradrenaline. Reserpine interferes with the storage of noradrenaline, and in the concentrations used is not believed to have many other actions (Trendelenburg, 1972). Thus in tissues in which the storage of noradrenaline was impaired, the response to histamine did not show as great an increase in sensitivity as untreated tissues. Reserpinization of tissues would be expected to have no effect on sensitization, if there was either an increase in the number of receptors for histamine or an increase in the ability of receptors to interact with histamine. Similarly, it is difficult to see how reserpine could interfere with an increase in the concentration of histamine at the receptor, since histamine is not believed to be stored in sympathetic nerve terminals.

The possibility that histamine is causing release of other substances was also investigated. Atropine has no effect on the sensitization to histamine, so it is unlikely that histamine is causing release of acetylcholine. In any case, no parasympathetic innervation of rabbit portal vein has been observed. Similarly, the inability of indomethacin to influence the sensitization to histamine implies that this agent is not releasing or inhibiting the release of prostaglandins.

Treatment of tissues with 6-OHDA seems to abolish the increase in sensitivity seen to histamine, while at the same time, the increased efflux of ^{3}H-noradrenaline caused by histamine is also abolished by 6-OHDA. These results suggest that the increase in sensitivity to histamine is in fact related to an increasing release of noradrenaline from the sympathetic nerve terminal. The two possible mechanisms of such a process were described in section I-A of this chapter. The tyramine-like action is inhibitible by cocaine, but efforts to block the increase in sensitivity to histamine with cocaine were unsuccessful. There are a number of possible reasons for this; for example, cocaine could be having a direct sensitizing effect on the response to histamine, and this could be masking the inhibition of the indirect effect produced by this agent. Trendelenburg (1972) has made the observation that failure to alter the response to sympathomimetic amines with cocaine does not necessarily mean that these agents are acting directly; it is possible that sensitization to the direct effects of an agent with both direct and indirect actions would offset any decrease in response due to inhibition of the indirect actions of these agents. It is unlikely that this is the true explanation for the failure of cocaine to prevent sensitization however, since the histamine response did not show the expected sensitization in tissues treated with 6-OHDA.

Cocaine does appear to have some effect on the sensitization seen to histamine though, since the maximum response of the first dose-response curve obtained after the addition of cocaine to the tissue is actually reduced compared to the initial maximum response. After this however, the tissues begin to sensitize in the same manner as normal tissues. In addition, the increase in the efflux of [^{3}H]-noradrenaline seen with histamine is significantly reduced in the presence of cocaine. These results suggest that cocaine has some effect on the release of noradrenaline caused by histamine, but it does not appear to be a particularly good antagonist of this response.

The other possibility is that histamine is causing release of noradrenaline by a DMPP-like mechanism. This mechanism is calcium-dependent, unlike the release caused by tyramine, and appears to cause depolarization of the nerve terminal and subsequent exocytosis of noradrenaline. This is believed to be the mechanism of action of nicotinic compounds whose noradrenaline-releasing actions can be blocked by hexamethonium, and which are believed to act via nicotinic receptors located on the sympathetic nerve terminal. Recent evidence has indicated that 5-HT may release noradrenaline by a similar mechanism in rabbit heart, and the release by both 5-HT and DMPP in that preparation can be blocked by colchicine, an agent known to inhibit exocytosis of noradrenaline, as well as by lowering the calcium concentration.

The sensitization of portal vein to histamine also appeared to be inhibited by methysergide, as did the sensitization to 5-HT. Since the concentration of methysergide used was very high, it is possible that this antagonist was having a non-specific effect on the preparation. However, it is also possible that 5-HT itself or receptors for this agonist are involved somehow in the mechanism of sensitization to histamine. If histamine is causing release by a 'DMPP-like' action, the receptor mediating this effect could be similar to the 5-HT 'D' receptor.

Although the discussion above was restricted to the mechanism of sensitization of histamine, the responses to 5-HT, betazole and tolazoline also showed various degrees of sensitization as well. This implies that if all these agents act by the same mechanism in causing noradrenaline release, the mechanism is relatively non-specific.

It is possible that if all act on the same post-synaptic receptors in high concentrations, then they could all also be acting through the same pre-synaptic receptors to cause release. However at this point in time, the small amount of evidence available concerning the mechanism by which these agents increase in sensitivity makes it very difficult to generalize.

IV CONCLUSION

The main conclusions arrived at in the preceding discussion are as follows: histamine appears to be acting directly on smooth muscle in rabbit portal vein to produce a response that can be blocked by phentolamine. The receptor at which histamine is acting can be characterized as a non-specific amine receptor, since 5-HT and noradrenaline also appear to be acting through this structure; it is probably a further sub-type of adrenergic receptor, since noradrenaline acts as a full agonist, and phentolamine, azapetine and dibozane all antagonize responses produced through it.

The increasing sensitivity of rabbit portal vein to histamine with time appears to be mediated indirectly through this receptor. The sensitization seems to result from the release of noradrenaline by histamine, but the mechanism by which this release occurs is not known at the present time. It is also not clear whether the responses to 5-HT, betazole and tolazoline sensitize by a similar mechanism.

The extremely high concentrations of histamine required to produce a response in rabbit portal vein make it unlikely that, under normal circumstances, histamine interacts with these receptors _in vivo_. However, as mentioned in the introduction, in conditions such as anaphylactic shock, the blood levels of histamine can reach high concentrations, and under these circumstances, histamine might then combine with these receptors and cause constriction of the portal vein. A similar insensitivity to low doses of histamine occurs in the portal veins of dogs (Greenway and Oshiro, 1973), rats and guinea pigs (unpublished observations). In the dog, however, the histamine response is believed to be mediated through classical H_1 receptors.

The presence of a receptor able to interact with three agonists normally considered to act on specific receptors, makes this preparation unusual. However, as mentioned in previous chapters, many drugs, particularly antagonists, have been found to interact with more than one species of receptor. For instance, α-adrenergic antagonists are able to block the histamine response and antihistamines are known to have anti-cholinergic activity (Douglas, 1975). β-adrenoceptor antagonists have been shown to block the respones to 5-HT, acetycholine and histamine (Furchgott, 1972). Cyproheptadine is equally effective at antagonizing the responses to histamine and 5-HT (Douglas, 1975). Recently it has been found that some agonists can react with more than one type of receptor, depending on the tissue being examined. 5-HT is a well known example: it is able to act both pre-and post-synaptically, and on 5-HT receptors as well as on α-adrenoceptors. Tolazoline can act as an α-adrenergic antagonist, or as an α-adrenoceptor or H_2 receptor agonist. It therefore appears that receptors are not the rigid unyielding structures they are sometimes conceived to be. This view is supported by the finding that alterations in temperature and in the metabolic environment can result in a change in the characteristics of receptors within a tissue. The apparent interconversion of α- to β-adrenoceptors and H_1 to H_2 receptors which has been previously mentioned, together with other evidence as described above, has led Kunos (1978) to state:

> "The large body of evidence allowing pharmacological classification of adrenoceptors has created an illusion of morphological reality in the minds of many pharmacologists. Although the nature and localization of α- and β-adrenoceptors ar still unknown, a strong implication of functionally and morphologically distinct, well-defined static membrane structures has been inherent in many studies. In the past few years a more dynamic image of receptors, with considerable molecular plasticity has emerged...".

Considering the current concepts of membranes as dynamic, fluid structures, it is not surprising that receptors have come to be viewed as dynamic structures also, subject to alterations by changes in their enviroment and by drugs. Under these circumstances it is possible to visualize the receptor present in portal vein, not so much non-specific, as able to adapt its conformation to the structures of various drugs. At the same time, the receptor does not seem to be entirely non-discriminating - it does not respond to all agonists, nor is it blocked by all antagonists. It is possible to continue speculating endlessly about the nature of such a receptor, and it's relation to other receptors in this and other tissues. For instance, it may be that rather than having a large number of α-adrenoceptor sub-types which are distinct from one another, these structures, including the one in portal vein, are all different conformations of the same macromolecule. The conformation of this structure could be controlled by the immediate enviroment of the receptor, i.e., the lipid matrix of the membrane surrounding the receptor, by the hormonal state of the animal and by other as yet undiscovered factors.

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APPENDIX

I DRUGS USED

Histamine Dihydrochloride (Sigma)

(-) - Noradrenaline Bitartrate (Sigma)

Acetycholine Bromide (BDH Ltd.)

Tyramine Hydrochloride (Mann Research)

5-Hydroxytryptamine Creatinine Sulfate (Sigma)

Chlorpheniramine Maleate (Schwarz/Mann Co.)

Diphenhydramine Hydrochloride (Sigma)

Phentolamine Methanesulfonate (Ciba)

Metiamide (SKF)

Methysergide Bimaleate (SKF)

6-Hydroxydopamine Hydrobromide (Regis)

Cocaine Hydrochloride (BDH Ltd.)

L-Ascorbic Acid (MCB)

Reserpine (Sigma)

Betazole Hydrochloride (Lilly)

Tolazoline (Ciba)

4-Methyl-Histamine (SKF)

N-Methyl-Histamine (SKF)

Ethyl-2-Pyretamine Hydrochloride (Midland Oil Distilleries Ltd.)

Antazoline Hydrochloride (Ciba)

Azapetine Phosphate (Roche)

Dibozane (McNeill)

Tropolone (Aldrich)

Pargyline Hydrochloride (Abbott Laboratories)

Phenoxybenzamine (SKF)

Atropine Sulfate (Matheson, Coleman and Bell)

Guanethidine Sulfate (Ciba)

Indomethacin (Sigma)

II COMPOSITION OF SOLUTIONS

KREBS' SOLUTION

NaCl	(113.0 mM)
KCl	(4.7 mM)
$CaCl_2$	(2.5 mM)
KH_2PO_4	(1.2 mM)
$MgSO_4$	(1.2 mM)
$NaHCO_3$	(2.5 mM)
Dextrose	(11.5 mM)

FLUOR

Naphthalene	(100 gm/1)
Omnifluor	(8 gm/1)
1, 4 Dioxane	(720 ml/1)
Toluene	(135 ml/1)
Methanol	(45 ml/1)